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DOCTOR OF MEDICINE

The roles of genetics and glycaemic control in the development of LVH and Heart Failure in Type 2 Diabetes

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MD title

The roles of genetics and
glycaemic control in the
development of LVH and Heart
Failure in Type 2 Diabetes

February 2015

Helen Parry

For the degree of MD

University of Dundee

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Abbreviations

A	Adenine
ACCORD	Action to Control Cardiovascular Risk in Diabetes Study Group
ADVANCE	Action in Diabetes and Vascular Disease
AGEs	Advanced Glycation End-products
ARIC	Atherosclerosis Risk In Communities
ASE	American Society of Echocardiography
BMI	Body Mass Index
BNF	British National Formulary
BP	Blood Pressure
BSE	British Society of Echocardiography
C	Cytosine
CAD	Coronary Artery Disease
CAN	Cardiac Autonomic Neuropathy
CI	Confidence Interval
CHARGE	Cohorts for Heart and Aging Research in Genome Epidemiology
CHI	Community Healthy Index
2D	Two-dimensional
DCCT	Diabetes Control and Complications Trial
DARTS	Diabetes Audit and Research in Tayside, Scotland

DCM	Dilated Cardiomyopathy
EA	Effect Allele
EAF	Effect Allele Frequency
ECG	Electrocardiogram
EDIC	Epidemiology of Diabetes Interventions and Complications
eGFR	estimated Glomerular Filtration Rate
G	Guanine
Go-DARTS	Genetics of Diabetes Audit and Research in Tayside, Scotland
GRO	General Registrar's Office
HbA1c	Glycosylated Haemoglobin
HbA1C_MEAN	Intra-individual mean of HbA1c value
HbA1C_SD	Standard Deviation intra-individual HbA1c values
HCM	Hypertrophic Cardiomyopathy
HIC	Health Informatics Centre
HF	Heart Failure
HMM	Hidden Markov Model
IBD	Identical by Descent
ICD	International Classification of Disease
IVS	Interventricular Septum
LV	Left Ventricle

LVH	Left Ventricular Hypertrophy
LVIDD	Left Ventricular Internal Diameter in Diastole
LVPW	Left Ventricular Posterior Wall
MAF	Minor Allele Frequency
MAGIC	Meta-Analyses of Glucose- and Insulin-related traits Consortium
MEMO	Medicine and Medicines Monitoring Unit
M_HbA1C	Weighted Mean HbA1C
NA	Not Applicable
NHS	National Health Service
OR	Odds Ratio
PSD	Practitioner Service Division
P-value	Probability value
PVD	Peripheral Vascular Disease
RCTs	Randomised Controlled Trials
Ref	Reference
ROS	Reactive Oxygen Species
RWT	Relative Wall Thickness
SBP	Systolic Blood Pressure
SCI-DC	Scottish Care Information-Diabetes Collaboration
SE	Standard Error

SERCA	Sarcoendoplasmic Reticulum Ca ²⁺ ATPase
SIGN	Scottish Intercollegiate Guidelines Network
SMR	Scottish Morbidity Record
SD	Standard Deviation
T	Thymine
T2DM	Type 2 Diabetes Mellitus
TZDs	Thiazolidinediones
UK GPRD	United Kingdom General Practice Research Database
UKPDS	UK Prospective Diabetes Study Group
VADT	Veterans Affairs Diabetes Trial
VEGF	Vascular Endothelial Growth Factor
WTCCC 2	Wellcome Trust Case Control Consortium 2
WTTCCC	Wellcome Trust Type 2 diabetes Case Control Consortium
Yrs	Years

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Declaration by Candidate

I declare that I am the author of this thesis. All references have been consulted. The thesis is my own work and has not been previously submitted for a higher degree.

Helen Parry

Declaration of the Supervisor

I certify that Helen Parry has completed the equivalent of six terms of research and has fulfilled the conditions of the University of Dundee so that she is qualified to submit this thesis in application for the degree of MD.

Professor Chim Lang

Professor Colin Palmer

Work contributed by the candidate

All data from the Go-DARTS study and the Tayside echocardiography database were cleaned by the candidate. The Tayside echocardiography database was established and validated by Dr Douglas Elder. Cleaning and imputation of the genetic typing data from Go-DARTS was performed prior to this analysis. Data linkage between all Go-DARTS files and the Tayside echocardiography database was performed by the candidate. Identification and extraction of heart failure cases, non-heart failure controls, left ventricular hypertrophy cases and non-left ventricular hypertrophy controls was performed by the candidate.

The SAS programming for calculation of weighted mean HbA1C was written by Dr Louise Donnelly and the SAS programming for the analysis looking at the importance of intra-individual HbA1C variation was written by Dr Harshal Deskmukth. Analyses using SNPTTEST were jointly performed by the candidate and Dr Natalie van Zuydam. All other analyses including extraction of values for covariates, genotypic risk scores and meta-analyses were performed by the candidate.

Summary

Cardiovascular disease is the leading cause of morbidity and mortality in diabetes. Not everyone with diabetes develops either LVH or HF, proposed factors influencing this include glycaemic control and genetic factors. No studies to date have looked at the genetics of LVH and HF specifically in T2DM.

This thesis investigates the importance of glycaemic control and genetic factors in HF and LVH in T2DM. Data from patients with T2DM in the Go-DARTS study were used to identify individuals with HF, non-HF controls, individuals with LVH and non-LVH controls. Weighted mean HbA1C was calculated for each of them. Logistic regression analysis and proportional hazard regression analysis were performed to investigate whether glycaemic control was independently related to LVH and HF. Genetic typing for published loci associated with glycaemic control was performed and included in the proportional hazard regression analysis. Genotyping for published SNPs associated with LVH was also performed and included survival analysis looking at LVH.

Proportional hazard regression analysis showed weighted mean HbA1C $\geq 8\%$ was associated with LVH (HbA1C ≥ 8 to 9% HR 1.25, CI 1.04-1.50, HbA1C ≥ 9 to 10% HR 1.64, CI 1.29-2.09, HbA1C $\geq 10\%$ HR 1.80, CI 1.32-2.44, all p-values <0.05) and also demonstrated weighted mean HbA1C $<6\%$ was associated with LVH in T2DM (HR 1.95, CI 1.57-2.43, p-value <0.05). Two out of 9 published SNPs were associated with LVH in our cohort with T2DM: rs17132261 and rs2292462 (p-value <0.05).

Proportional hazard regression analysis showed HbA1C $\geq 8\%$ was associated with HF development and HbA1C $<6\%$ was also associated with HF development in T2DM (HbA1C $<6\%$ HR 2.2, CI 1.7-2.9, ≥ 8 to 9% HR 1.6, CI 1.2-2.0, ≥ 9 to 10% HR 2.5, CI 1.8-3.4 and weighted mean HbA1C $\geq 10\%$ HR 4.82, CI 3.6-7.0, all p-values <0.05). Conditional logistic regression analysis also showed 3 SNPs previously associated with fasting glucose were associated with HF development here (rs560887, rs7944584 and rs10885122).

These results suggest glycaemic variation and genetic factors are important factors in HF and LVH in T2DM.

Chapter 1: Introduction

1.1 Current trends in T2DM

Diabetes is a global concern. It is both common and associated with high levels of morbidity and mortality. Over 300 million people worldwide suffer from diabetes (1). Recent estimates showed the world prevalence of diabetes would increase from 6.4% in 2010 to 7.7% in 2030 among adults 20-79 years of age (2). In the UK, 3 818 545 individuals had diabetes in 2010-2011, of which 3 419 727 had type 2 diabetes mellitus (T2DM) (3). In Scotland alone, prevalence of diabetes has risen from 5.2% in 2003 to 9.4% in 2008 according to the Scottish Diabetes Survey (4).

The high prevalence of diabetes has led to high levels of mortality and morbidity. Estimates for 2010 indicated 6.8% of global mortality was attributable to diabetes. Diabetes accounted for 6% of adult deaths in Africa and 15.7% in North America, illustrating that although diabetes is a global issue, it is currently most prominent in the West (5). However, this may change in the future as developing countries become more westernised.

1.2 Cardiovascular Disease in T2DM

Cardiovascular disease is the leading cause of morbidity and mortality in patients with diabetes, making T2DM a great public health concern (6)(7)(8). Cardiovascular disease appears on more than two thirds of death certificates for diabetic patients aged 65 years and over (9). This is unsurprising since cardiovascular disease in diabetes promotes morbidity and mortality through heart failure and coronary artery disease.

Many studies have confirmed that cardiovascular disease is the leading cause of death in individuals with diabetes. In the Verona study, records from diabetes clinics, family physicians and prescribing data were used to identify individuals resident in Verona, Italy, who had type 2 diabetes in 1986. Their live status was ascertained 10 years later and cause-specific mortality rates were compared and cardiovascular disease was the leading cause of death as tabulated below (10). Similarly, Roper and colleagues performed a population cohort study, following over four thousand individuals with

diabetes over a five year period in the North East of England. Cardiovascular disease was also the leading cause of death in this population with deaths from ischaemic heart disease exceeding deaths from cerebrovascular disease (11). In the WHO Multinational Study of Vascular Disease in Diabetes (WHO MSVDD), cardiovascular disease accounted for 52% of all deaths in T2DM (12).

Table 1.1 Cause-specific mortality in the Verona study

Cause of death	N (%)
Cardiovascular	974 (42)
<i>Ischaemic heart disease</i>	<i>343 (15)</i>
<i>Stroke</i>	<i>235 (10)</i>
<i>Other CVD including HF</i>	<i>398 (17)</i>
Neoplasia	517 (22)
Diabetes-related	324 (14)
GI disease	134 (6)
Other natural causes	250 (11)
External causes	48 (2)
Unknown causes	81 (3)
Total	2328

Table adapted from Brun *et al*, 2000 (10)

1.2.1 Coronary artery disease (CAD) in T2DM

Both CAD and HF are common in T2DM; almost 25% of all diabetic individuals older than 35 years have some manifestation of CAD (9) and studies suggest 19-26% of diabetics also suffer from HF (13). Data from the Framingham study highlighted the association between T2DM and CAD many years ago (14,15). T2DM is now regarded as an independent risk factor for the development of CAD and associated with a more aggressive variation of the disease (9,16). One study suggested that diabetic patients without previous myocardial infarction have as high a risk of future myocardial

infarction as non-diabetic patients with previous myocardial infarction, bearing testimony to the extent of increased risk of CAD (7).

1.2.2 Heart Failure (HF) in T2DM

HF is also common in T2DM as demonstrated in a retrospective study of around 8231 individuals with T2DM and 8845 individuals without T2DM of similar age and matched for gender. Cox regression analysis showed individuals with T2DM were much more likely to develop HF than the non-diabetic individuals and CAD was an important predictor of HF (17). Taking the data outlined above into account, it seems logical that T2DM increases the risk of HF development by increasing the likelihood of CAD. However, T2DM still increases the risk of HF development even when CAD, hypertension and obesity are accounted for, implying a direct effect on the myocardium (18,19). The term 'diabetic cardiomyopathy' is frequently used to describe myocardial disease in T2DM occurring independently of CAD and the vascular risk factors listed above (20).

1.3 Diabetes and HF and "Diabetic Cardiomyopathy"

1.3.1 Epidemiology

Diabetic cardiomyopathy specifically refers to the development of HF in diabetes in the absence of any other identifiable cause, such as CAD. However, the term "diabetic cardiomyopathy" is also often used in a more general sense to refer to myocardial pathology in diabetes. Myocardial pathology in diabetes may take multiple forms including HF and left ventricular hypertrophy (LVH). HF is common in T2DM; studies suggest 19-26% of patients with diabetes mellitus also suffer from HF (13,21). T2DM is a major risk factor for the development of HF. Some of the earliest evidence for this came from the Framingham study: HF development was 6-fold greater in diabetic males and 8-fold greater in diabetic females than in their non-diabetic counterparts (22). NHANES and the Cardiovascular Health Study provided further evidence for T2DM as an independent risk factor for HF development (18,19). Around 0.3-0.5% of the general population have both HF and T2DM (23). Diabetes remains an independent predictor of LVH as well as HF, even when obesity and

hypertension are accounted for (24,25). Although supportive data is less plentiful than for the link between HF and diabetes, there is a general consensus between studies that diabetes is an independent risk factor for the development of LVH. One study quoted 26% of normotensive patients with diabetes have LVH on echocardiography in the context of CKD (26).

1.3.2 Types of HF in DM: systolic HF versus Heart Failure with Preserved Ejection Fraction (HFPEF)

Investigation into HF in T2DM has mostly focused on left ventricular systolic dysfunction (LVSD). However, HFPEF is becoming an increasingly recognised issue. Recent research has shown a significant association between T2DM and HFPEF and studies have shown almost half of patients with T2DM have diastolic dysfunction (27,28).

The association between T2DM and HFPEF appears to hinge on the prevalence of LVH in T2DM. An early study looking at cardiac dimensions and function in hypertensive patients with T2DM found that co-existent T2DM and hypertension were associated with increased LV mass and impaired E/A ratio, indicating diastolic dysfunction. This relationship was enhanced by the co-existence of urinary albumin, another marker of end-organ damage in diabetes (29). Another recent study looked at the impact of T2DM on cardiovascular geometry and diastolic dysfunction in chronic kidney disease. T2DM was associated with both increased wall thickness and increased relative wall thickness. Diastolic dysfunction, identified by lower E prime (30), was also significantly more common in T2DM, but this association lost statistical significance following correction for LV wall thickness (28).

Further work looking at the relationship between LVH in T2DM and diastolic dysfunction involved performing echocardiography in a group of patients with no cardiac symptoms and normal BNP levels. They found 51% of these individuals had LV diastolic dysfunction and LVH was significantly more common in these individuals with diastolic dysfunction (31).

The above evidence strongly suggests the association between T2DM and diastolic dysfunction is at least partly mediated through the increased prevalence of LVH in diabetics.

1.3.3 Aetiology of HF and LVH in T2DM

The aetiology of HF and LVH in T2DM is complex (Figure 1.1). Multiple studies have demonstrated a link between CAD and HF in T2DM (17,32,33) and it has long been recognised that myocardial ischaemia, particularly infarcted myocardium, leads to reduced cardiac pump function and HF development. However, as stated above, there is increased incidence of HF in T2DM even when CAD is taken into account (18,34).

Similarly, hypertension has been associated with the development of HF and LVH. Hypertension and obesity are both common in T2DM and both promote LVH (34–36), so it appears feasible that LVH in T2DM is caused by hypertension and obesity rather than a direct action on the myocardium.

However, diabetes remains an independent predictor of LVH even when obesity and hypertension are accounted for (24,25). One study quoted 26% of normotensive patients with diabetes have raised left ventricular mass index on echocardiography (26). This indicates T2DM promotes HF and LVH independently of hypertension, obesity and CAD.

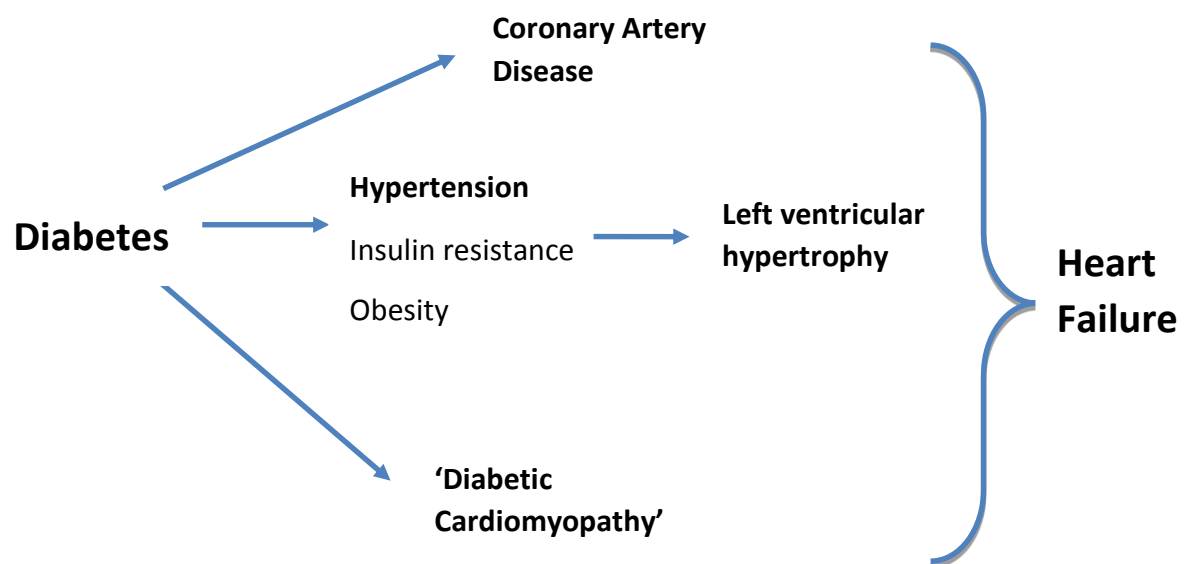


Figure 1.1: The interplay of aetiological factors in the development of HF in T2DM

T2DM appears to have a direct effect on the myocardium. T2DM is likely to act directly on the heart through a variety of mechanisms. Animal models suggest diabetes is associated with increased

production of reactive oxygen species leading to myocardial fibrosis and reduced contractility that can be ameliorated by antioxidants (37,38). Similarly, advanced glycation end products accumulate in the extracellular matrix resulting in myocardial stiffness and altered contractility (39,40). The myocardium additionally suffers from lipotoxicity as a result of fatty acid accumulation and cardiac myocyte degeneration and apoptosis reduce effective contractility (41). These aetiologies may promote the development of both HF and LVH.

Endothelial dysfunction appears to promote reduced coronary flow reserve in LVH as suggested by the reduced coronary artery vasodilator response to dipyridamole in LVH (42). This is supported by the improvement in microvascular dysfunction in patients with T2DM through endothelin A receptor blockade (43). Increased production of endothelial progenitor cells in LVH has been shown in animal models of pressure-overloaded LVH where pressure-overload led to up regulation of circulating progenitor cells, mirrored in the bone marrow and spleen (44). This leads to increased diffusion distance from capillaries to myocytes and contractile reserve is diminished (45), leading to ischaemia and ultimately to HF.

Other pathological processes directly affecting the myocardium relate to ion handling. Animal models have also shown reduced expression of the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) results in altered sodium and calcium handling, further altering contractility (46). Lastly, cardiac autonomic neuropathy (CAN) may be another contributory factor (47). CAN affects coronary blood flow and alters contractile function of the myocardium. Altered sympathetic tone has been associated with reduced vascular elasticity and increased peripheral resistance as described by Pappachan *et al* (48).

Indirect mechanisms also promote cardiac pathology in diabetes. Vascular pathology be important even in the absence of CAD on angiography. Reduced vascular endothelial growth factor (VEGF) levels reduce myocardial angiogenesis in T2DM as shown in the rat model of diabetes (49). Human studies looking at coronary flow reserve have also shown increased prevalence of microangiopathy

in T2DM (50). Microangiopathy was shown to promote HF in the absence of significant disease of the epicardial coronary arteries (51). LVH is also associated with reduced coronary flow reserve.

Figure 1.2 summarizes the likely pathophysiological processes acting both directly on the myocardium and indirectly for the development of HF in DM.

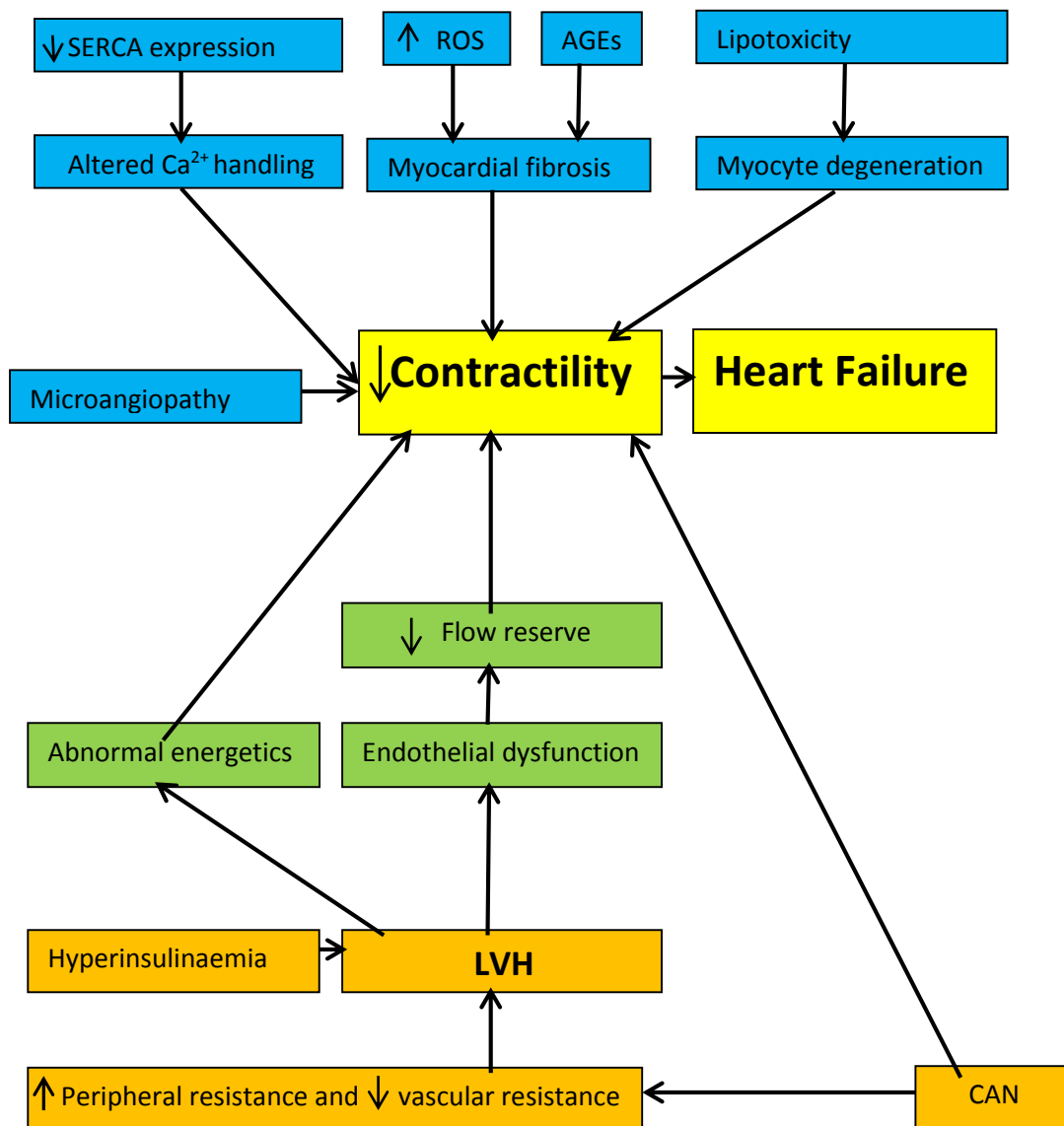


Figure 1.2: Pathophysiology of diabetic cardiomyopathy. Adapted from MacDonald M R *et al* (23).

There is significant evidence that LVH itself promotes HF development: multiple studies have demonstrated progression from LVH to HF (52–54). One such study showed older people with

persistent or new ECG features of LVH have higher incidence of HF development and tend to develop HF more quickly than those without LVH on ECG (53). In a more recent study, baseline echocardiogram and ECG were performed in 922 hypertensive patients who were then followed-up for 4.8 years to evaluate the development of HF. The presence of LVH, according to both ECG and echocardiographic criteria, predicted hospitalisation for HF independently of other covariates including ischaemic heart disease (52).

Various pathophysiological mechanisms accounting for the progression from LVH to HF have been proposed. Maslov *et al* investigated the role of abnormal myocardial energetics. They point out magnitude of pressure overload, genetic, neuroendocrine and mechanical remodelling contribute to the progression of LVH to HF and that increase in energy demand underlies many of these contributing factors. In mouse hearts, energetics abnormalities occurred early in pressure-overload LVH. Early energetic changes predicted the extent of subsequent left ventricular remodelling; consistent with the hypothesis altered energy metabolism in LVH begets HF (55).

Subsequent work has looked at T-tubule remodelling in the progression of LVH to HF. T-tubules are responsible for rapid electrical excitation and co-ordinated muscle contraction through synchronous triggering of calcium release from the sarcoplasmic reticulum. Animal models showed T-tubule remodelling began during compensated LVH prior to echocardiographically detectable systolic dysfunction and continued to progress along with decline in LV function. T-tubule remodelling may be an early event marking the transition from fully compensated LVH to decompensated HF (56). As previously mentioned, LVH is associated with reduced coronary flow reserve (42,57); which may provide another potential mechanism for LVH progression to HF.

1.3.4 Glycaemic control and the development of LVH and HF

If diabetes causes LVH and HF, how important is glycaemic control in this process? HbA1C is generally regarded as the most accurate measure of medium-term glycaemic control. Glucose within the blood stream adheres to haemoglobin within red blood cells, forming glycated

haemoglobin. The relative concentration of glycated haemoglobin is given by HbA1C measurements, which indicate glycaemic control over the preceding 2-3 months, reflecting the average life-span of red blood cells. However, the optimum target HbA1C in T2DM is still debated. The SIGN guidelines state 7.0% is a reasonable target in T2DM and a target of 6.5% may be appropriate at diagnosis. The NICE guidelines refer to a 'general' target of 6.5%. Diabetes UK suggest HbA1C should ideally be <7.5% within a year from diagnosis. Both SIGN and NICE guidelines, and Diabetes UK recommendations, state target HbA1C should be agreed on an individual basis, balancing the risks of microvascular and macrovascular disease associated with hyperglycaemia against the risks of hypoglycaemia and weight gain associated with strict glycaemic control.

The frequency with which HbA1C measurements are taken are also generally decided on an individual basis. Diabetes UK recommend patients with newly diagnosed diabetes are reviewed regularly until blood glucose is stabilised. Thereafter, HbA1C should be measured at least every 6 months, although in young adults with poor glycaemic control HbA1C should be monitored more closely.

In view of the above, most studies looking at how glycaemic control influences outcome in T2DM use HbA1C measures for assessing glycaemic control. However, there has been very little research looking at whether poor glycaemic control promotes LVH development. Medline search revealed 2 studies looking at glycaemic control and LVH. A cross-sectional study in which echocardiography was performed on 262 patients with T2DM who were normotensive and had normal urinary albumin levels. Forty-four per cent of these individuals met echocardiographic criteria for LVH. Logistic regression analysis showed glycaemic control, BMI and log-urinary albumin excretion were all associated with LVH in this population (58). A second study was performed looking at a cross-section of patients on maintenance haemodialysis. They found glycaemic control was generally poorer in patients on maintenance haemodialysis and glycaemic control was associated with cardiac

complications including LVH (59). While this was a useful starting point, these findings must be verified in larger studies.

In contrast, there is a large body of literature looking at whether glycaemic control effects the development of HF. Observational studies have provided a useful evidence base. Work using the Kaiser Permanente Diabetes Registry showed HbA1C>7% was associated with increased risk of HF development in T2DM, where analysis was performed using a single HbA1C measure and HF development was defined as hospital admission with HF (60).

These results were supported by the ARIC study, which also found HbA1C>7% was associated with increased risk of HF development. This was true both in individuals with CAD and in those without CAD, indicating glycaemic control influences HF development beyond its influence on CAD development. This analysis also used a single HbA1C measure and defined HF as either hospital admission with HF or the appearance of HF on the death certificate (61). Most recently, a study using the Swedish National Diabetes Registry showed HbA1C>6% was associated with increased risk of HF development. This analysis was performed using updated mean HbA1C, arguably more reflective of long term glycaemic control than a single measure, and HF was defined by hospital admission (62).

Four major randomized controlled trials (RCTs) have looked at how glycaemic control influences the development of HF in T2DM. They have yielded inconsistent results, conflicting both with one another and with results from the observational studies described above. The ACCORD study, enrolled 10 251 individuals with T2DM, poor glycaemic control and evidence of cardiovascular disease. They were randomized to intensive therapy with target HbA1C<6% or standard therapy with target HbA1C 7-7.9%. The study was terminated prematurely due to increased mortality in the intensive therapy group (HR 1.22, CI 1.01-1.46). There was no significant difference in the rate of HF development between the two groups (63).

The ADVANCE and VADT trials also failed to demonstrate any benefit from improved glycaemic control. The ADVANCE study included 11140 patients with T2DM, either macrovascular or microvascular disease and at least one other vascular risk factor. These patients were randomized to intensive glycaemic control, aiming for HbA1C<6.5%, or standard therapy. Individuals in the intensive control group were commenced on gliclazide (30-120mg daily) and discontinued any other sulphonylurea. The gliclazide dose was titrated according to glycaemic control with the sequential addition of metformin, thiazolidinediones, acarbose and insulin. Individuals in the standard therapy group taking gliclazide prior to study enrolment were required to substitute it with another sulphonylurea. Although intensive glycaemic control significantly reduced the risk of the composite end-point (major microvascular or macrovascular event, HR 0.90, CI 0.82-0.98) there was no significant difference in mortality between the two treatment arms (HR 0.93, CI 0.83-1.06) and no significant difference in the risk of HF development (64).

The VADT study yielded similar results to the ADVANCE study. One thousand seven hundred and ninety-one patients with HbA1C>7.5% were enrolled and assigned to intensive versus standard care. All patients with BMI >27 were commenced on metformin and rosiglitazone and all patients with BMI <27 were commenced on glimepiride and rosiglitazone. Patients in the intensive therapy group were put on maximum doses at the beginning of the study and those in the standard therapy group were placed on half maximum doses. Median HbA1C 6.9% was achieved in the intensive therapy group and 8.4% in the standard therapy group. There was no significant difference between time to death ($p=0.32$) or first cardiovascular event (HR 0.88, CI 0.74-1.05) between the 2 groups. The authors point out there were fewer events in both groups than predicted, which may have meant the study was under-powered to detect a small difference in risk (65). Both the ADVANCE and VADT trials were under-powered to detect a significant difference in the rates of HF development.

The UKPDS trial, although not strictly speaking an RCT, yielded more positive results. Newly diagnosed type 2 diabetics ($n=3867$) were randomized to intensive therapy, with either

sulphonylurea or insulin, or to conventional therapy. All-cause mortality was reduced by 6% in the intensive therapy group, although there was no significant difference in HF rates between the 2 groups (66).

Why did the RCT results largely conflict with the observational study results? There are many potential explanations. The observational studies looked at all individuals who developed HF after their diagnosis of T2DM. The ACCORD and VADT studies specifically enrolled individuals with poor glycaemic control (64,65). By definition, these patients had not responded to the first-line glucose lowering measures. Arguably, they were a self-selecting group who would require multiple high-dose second and third line therapeutic agents, increasing their risk of developing side effects.

Sulphonylureas, TZDs and insulin have all been implicated in HF in T2DM. A recent study showed increased all-cause mortality in patients treated with sulphonylureas relative to metformin. The difference between rates of HF development in first generation sulphonylureas (e.g. tolbutamide, tolazamide) compared to metformin was not statistically significant. However, second generation sulphonylureas (e.g. gliclazide, glibenclamide, glipizide) were associated with increased rates of HF development relative to metformin (67). Meta-analysis of available clinical trials data showed rosiglitazone was associated with increased risk of HF development and rosiglitazone and pioglitazone are both known to promote fluid retention (68,69). Trials investigating the consequences of insulin therapy have looked at differences in mortality rates and all cardiovascular events rather than specifically HF development. One observational study demonstrated a graded increase in the risk of mortality and cardiovascular events related to insulin-exposure (70). Another study found insulin use was associated with worse prognosis in HF (71). The UKPDS study showed a reduction in cardiovascular events with combination sulphonylurea/insulin therapy, but this was non-significant (66). The body of evidence suggests increased mortality with tight glycaemic control in ACCORD may be partly accounted for by adverse drug effects (63).

‘Metabolic memory’, the concept that the early glycaemic environment in diabetes is remembered by target organs, may also partly explain the conflicting results above. Evidence for this phenomenon initially came from the DCCT/EDIC studies where patients with type 1 diabetes were randomized to intensive or conventional glycaemic control strategies. The reduction in microvascular complications and cardiovascular events in the intensive therapy group resulted in termination of the trial after 6.5 years and all patients were placed on intensive therapy. Later follow-up showed the patients on conventional therapy had increased carotid intimal thickness (used as a proxy for macrovascular disease) relative to the intensive therapy group, despite practically equivalent HbA1Cs across the two groups (72–74).

Evidence from UKPDS also supported the concept of ‘metabolic memory’ in T2DM. Following study completion, all patients and clinicians were advised to lower blood glucose as much as possible. The difference between mean HbA1C in the intensive control group and the conventional group was lost by 1 year following study termination. Despite this, individuals initially receiving intensive therapy continued to have reduced rates of microvascular disease, all-cause mortality and myocardial infarction than those in the initial conventional therapy group (75). The molecular basis of ‘metabolic memory’ is largely unknown, although oxidative stress and accumulation of advanced glycation end products may be relevant (72). It is possible that an RCT looking at intensive versus standard glycaemic control in newly diagnosed diabetics only would yield different results to the RCTs detailed above, potentially demonstrating the benefit of early intensive glycaemic control.

HbA1C is arguably the most accurate reflection of medium-term glycaemic control. However, it does not reflect the intra-individual range of plasma glucose nor does it provide information regarding the number of hypoglycaemic events an individual has had. All the above RCTs showed increased rate of hypoglycaemic events in the intensive control groups, which may promote increased mortality and HF development. It is universally recognised that hypoglycaemic events are dangerous in the short term, leading to reduced consciousness, coma and death if untreated. However, episodes of

moderate hypoglycaemia may also have long term effects. Hypoglycaemic events are pro-inflammatory, pro-thrombotic and atherogenic (76). Wright *et al* compared 16 type 1 diabetics and 16 non-diabetics during euglycaemia and hypoglycaemia. Platelet activation occurred after hypoglycaemia in both diabetics and healthy controls. Hypoglycaemia also triggered inflammatory processes and CD40 levels were maximally elevated 24 hours following hypoglycaemia (77). Similarly, Gogitidze and colleagues found moderate hypoglycaemia led to increased PAI-1, VEGF, vascular adhesion molecules and markers of platelet aggregation in both healthy volunteers and type 1 diabetics (78).

Comparable studies in patients with T2DM have not yet taken place. However, it seems likely this pro-thrombotic, pro-inflammatory and pro-atherogenic state resulting from hypoglycaemia played a part in the adverse outcomes associated with intensive glycaemic control in the studies above. If the frequency of hypoglycaemic events had been accounted for in these analyses, would the outcomes have been different?

The evidence discussed above indicates both hyperglycaemia and hypoglycaemia are implicated in HF development in T2DM. In view of this, the question is posed: do widely fluctuating blood glucose levels also increase the risk of cardiovascular disease in T2DM? Studies to date have investigated the importance of both diurnal variation in blood glucose levels (glycaemic variation) and longer-term variation in HbA1c. Plentiful evidence has supported an association between HbA1c variation and both mortality and development of nephropathy in type 1 and type 2 diabetes (79–83).

Research investigating the association between variation in blood glucose and cardiovascular disease has yielded some conflicting results. Juarez *et al* performed a retrospective cohort analysis on patients with poor glycaemic control; defined as HbA1c >9% in individuals with at least annual HbA1c measures for a minimum of 3 years. Patients were classified as having ‘good’ glycaemic control (mean HbA1c <7%), ‘poor’ glycaemic control (mean HbA1c >9%), ‘wide glycaemic variability’ (patients with HbA1c fluctuating from greater than 9% to less than 7% to greater than 9%) and ‘some

glycaemic variability' (those who did not fit into any of the above categories). Using a multivariate logistic regression model, they found 'wide glycaemic variability' was associated with CAD but not with HF (84).

Very few other studies have looked specifically at how variation in HbA1c influences the development of HF. Most research into the cardiovascular impact of wide variation in HbA1c has grouped coronary artery disease, stroke, HF and PVD together. In the FinnDiane study, 2 107 patients with type 1 diabetes had a median of 13 HbA1c measures over a median follow up period of 5.7 years. Mean and standard deviation were calculated from the HbA1c values. Higher mean HbA1c was associated with renal disease but not cardiovascular disease. However, greater intra-personal standard deviation in HbA1c values was associated with both renal and cardiovascular disease where cardiovascular disease was defined as myocardial infarction, coronary artery procedure (by-pass surgery or angioplasty), stroke (ischemic or haemorrhagic), limb amputation due to ischemia, or a peripheral artery procedure according to medical records reviewed both at baseline and follow-up (85).

A prospective cohort study on a similar theme looked at 8439 Chinese patients with T2DM, calculating HbA1c mean and standard deviation for each individual during follow up. Cox regression analysis showed both HbA1c mean and standard deviation were independently associated with both the development of chronic kidney disease (defined as eGFR<60) and the development of cardiovascular disease (defined as CAD, HF, stroke or PVD (86). In view of the above, although HbA1c variability appears to promote cardiovascular disease, there is currently insufficient evidence for an association specifically between HbA1c variability and HF.

1.4 Is there a genetic component to LVH, HF and diabetic cardiomyopathy?

Although LVH and HF are very common in patients with T2DM, they are not universal. Not all patients with T2DM develop LVH, and those developing LVH do so to varying degrees (25). Early studies showed the prevalence of LVH was between 15 and 20% in the general population (87).

The ASE categorise the varying degrees of LVH as mild, moderate and severe for all methods of defining LVH, including 2D thickness, relative wall thickness, overall LV mass, LV mass indexed to BSA and LV mass indexed to height (88). As pointed out by Ruilope and colleagues, increase in LVM directly correlates with cardiovascular risk and regression of LVH is associated with reduction in cardiovascular risk (89). Alternatively, LVH may be categorised according to pattern of LVH and LV mass relative to cavity size as eccentric dilated, eccentric non-dilated, concentric dilated and concentric non-dilated (90). Concentric LVH refers to increased LVM in the context of increased LVM/end diastolic volume ratio. Eccentric LVH refers to raised LVM in the context of normal LVM/end diastolic volume ratio. Both concentric and eccentric LVH are classed as dilated/non-dilated depending on the end diastolic volume.

Evidence of heritability of LVH

This heterogeneity is likely to have a partial genetic basis. LV mass heritability in non-diabetic subjects has been estimated through twin studies (91), studies in hypertensive siblings (92) and complex family studies (93,94). Maternally transmitted genetic susceptibility to LVH in T2DM has also been reported (95).

Glycaemic control appears to play a role in whether patients with T2DM develop HF, as stated above. However, it is unlikely to be the only determinant. Plentiful evidence suggests genetic factors have a role in HF development. The genetic basis of HF is a combination of the genetic variants associated with aetiological factors, including diabetes, and variants independently associated with heart failure. Estimates indicate around 30% of dilated cardiomyopathy appears to be familial (96,97). It is possible that genetic factors and glycaemic control not only work concomitantly to promote HF in T2DM, but may also be inter-twined.

1.4.1 Genetic basis of LVH

The genetic basis of LVH in both diabetics and non-diabetics has been investigated through candidate gene studies and genome wide association studies (GWAS). Candidate gene studies investigate whether variants within genes coding for proteins within the neuro-hormonal pathways involved in the pathophysiology of LVH predict LVH development.

The angiotensin converting enzyme (ACE) gene has attracted most attention, but research has yielded conflicting results. Early studies focused on hypertensive cohorts. Fu and colleagues looked at the relationship between single nucleotide polymorphisms (SNPs) in the bradykinin- receptor beta 2 gene and the insertion deletion ('I/D') variant of the ACE gene in 275 hypertensive patients and 441 controls. Neither SNP was associated with hypertension but both SNPs appeared to be involved in predicting LVH in patients who were already hypertensive (98). A separate study showed the ACE D/D genotype was associated also with increased risk of LVH post-myocardial infarction (99). However, one study focusing on patients with insulin resistance and diabetes did not found an association between LVH and the ACE I/D variant (100).

Research examining other candidate genes has also largely been limited to hypertensive patients rather than diabetics. Two projects looking at genetic variation in the G-protein beta-3 subunit have provided evidence the 825T allele partially predicts LVH development (101,102). Another study in hypertensive patients showed 2 SNPs within insulin-like growth factor 1 were associated with echocardiographically defined LVH (103). A large (n=1678) population based study in Augsburg, Germany, additionally provided relatively strong evidence variation in the ghrelin receptor gene predicted left ventricular dimensions independently of BMI and blood pressure (104).

Only 2 population based genome wide association studies (GWAS) have sought single nucleotide polymorphisms (SNPs) associated with LVH. Vasan *et al* compared cases of echocardiographically defined LVH with controls across 5 discovery cohorts (n=12 612) and 2 replication cohorts (n=4 094). They found multiple SNPs linked to left ventricular thickness, left ventricular mass and left

ventricular failure, but only replicated SNPs associated with increased left ventricular thickness and left ventricular systolic dysfunction (105). Shah *et al* performed a GWAS looking at 10 256 individuals from 3 population based cohorts, defining LVH based on ECG criteria. They discovered 12 single nucleotide polymorphisms (SNPs) associated with LVH, 3 of which were also significant in their replication cohort (n=11 777) (106).

These findings have not been reproduced in subsequent studies, although Shah and colleagues unsuccessfully tried to replicate their findings in the EchoGen consortium (106). No studies to date have identified genes predicting development of LVH specifically in patients with diabetes.

1.4.2 Genetic basis of HF

Evidence of heritability

Plentiful evidence supports a partial genetic basis for HF (107–111). The genetic basis of HF may be divided into genetic variants directly promoting HF and those associated with the causes of HF.

There are many different causes of HF, most of which have a genetic component. Coronary artery disease (CAD), valvular disease, hypertension and diabetes are the most common aetiological factors. There is a wealth of evidence showing CAD has a partial genetic basis (112). This is unsurprising since the independent risk factors for ischaemic heart disease, including hypertension (113), hyperlipidaemia (114) and diabetes mellitus (115), all have genetic components. Aside from a small group of specific genetic syndromes, there is less evidence to support a genetic basis for valvular pathology, although fewer studies have examined this issue.

However, studies referenced above have demonstrated a genetic component to dilated cardiomyopathy, independent of its aetiological factors (107,110,116). Candidate genes within the sympathetic and rennin-angiotensin-aldosterone pathways have been investigated. Variants within the beta 1 and beta 2 adrenergic receptor genes have been associated with differences in sympathetic response and difference in beta blocker response in heart failure, but do not appear to

predict the development of heart failure (117–120). Similarly, another study showed a mutation in the angiotensin converting enzyme (ACE) was associated with variation in response to ACE inhibitors but did not predict heart failure development (121,122).

Candidate genes encoding proteins that make up the molecular structure of the myocardium have also been investigated. Genes encoding sarcomeric proteins have received particular interest: hypertrophic cardiomyopathy is caused by an array of different mutations within the genes encoding sarcomere proteins (123). Kamisago *et al* expanded on these findings by investigating the role of these variants in DCM. They looked at 21 kindreds in which DCM appeared to be inherited in an autosomal dominant manner. They found a missense mutation in the cardiac β - myosin heavy chain gene and a deletion in cardiac troponin T gene were predictive of early onset ventricular dilatation and diminished contractile function, frequently leading to HF (124).

Subsequent work has extensively explored the effect of variants encoding proteins within the sarcomere. Another study showed three mutations previously associated with HCM were found in DCM patients with no evidence of a preceding hypertrophic phase, indicating phenotypic plasticity (125).

Variants altering Z-disc protein structure and calcium handling have also been associated with both DCM and HCM (126). As detailed in the review by Bos and colleagues, there are 6 genes with variants associated with HCM coding for Z-disc proteins but only one of these variants has a minor allele frequency of >1%. This variant lies within the muscle LIM protein, an essential regulator of myogenic differentiation (127). A large study involving sequencing this gene in 400 DCM patients, 200 HCM patients and 500 controls discovered 3 different missense mutations in 3 unrelated individuals with HCM but none in DCM (126).

Recent studies examining the significance of variants in Z-disc proteins in DCM have looked at Bcl associated athanogene 3 (BAG3), which is a co-chaperone protein with anti-apoptotic function that localizes at the Z disc in striated muscle (128). Variation in genes for Z disc proteins are unlikely to

account for a significant proportion of HCM cases. Further work is needed to assess the frequency and significance of these mutations at the population level.

Further studies have explored the relevance of genetic variants influencing calcium handling.

Mutations in the phospholamban gene were associated with the development of heart failure (129).

This is unsurprising since phospholamban regulates the sarcoplasmic reticulum calcium-ATPase (130,131), thus controlling calcium handling and muscular contraction. However, phospholamban variants are only likely to account for approximately 0.4% of HF cases as these mutations are very rare.

Very few published GWAS have looked at HF development. Variants in the gene encoding BAG 3 were implicated in the development of DCM following 2 GWAS (111,132). Villard *et al* looked at a discovery cohort of 1179 DCM cases and 1108 control patients. Fourteen SNPs associated with HF were identified, 2 of which were replicated including rs2234962 within BAG3 on chromosome 10 as mentioned above. The second SNP, rs10927875, was found in HSPB7, which encodes a heat shock protein (111). Another SNP within HSPB7 was linked with HF in a previous association study in which a cardiovascular gene-centric 50 000 SNP array was used as a compromise between a candidate gene study and a GWAS (133). The heat-shock proteins are chaperones and have a protective function in cardiovascular disease. Plasma HSPB7 concentration has recently been shown to be elevated following acute MI in both murine and human models (134).

The CHARGE consortium also performed a GWAS meta-analysis looking at 2.5 million SNPs. They identified 2 SNPs associated with HF at the genome-wide significance level, although their relevance within cardiovascular disease is questionable. Rs1739843 does not lie within a specific gene and rs11172782 lies within LRIG3, part of a family of leucine-rich repeats and immunoglobulin-like domain proteins, which has been associated with multiple tumours including astrocytoma, pituitary adenoma and bladder cancer and has also been linked to psoriasis (135–137).

No studies to date have looked at the genetic basis of HF specifically in T2DM, which is surprising given the frequency with which both HF and T2DM occur together.

1.5 Genetic basis of glycaemic control

Early research looked for genetic variants predisposing to T2DM as a binary trait, but more recent work has looked at the continuous traits associated with glucose homeostasis. Fasting glucose and HbA1C have been studied as indicators of glycaemic control, with focus on GWAS rather than candidate gene studies (138). The Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) was formed to conduct large-scale meta-analyses to identify genetic variants associated with continuous diabetes-related traits in non-diabetic individuals (139). The MAGIC investigators performed a 2 stage association study with 21 discovery cohorts in 46 186 individuals for 2.5million SNPs to identify SNPs associated with fasting glucose. Lead SNPs identified in the discovery cohort were followed up in a second cohort including 76 558 individuals. New SNPs associated with fasting glucose were found in or near ADCY5, MADD, ADRA2, CRY2, FADS1, GLIS 3, SLC2A2, PROX1 and C2CD4.2. Relationships between SNPs previously associated with fasting glucose in genes GCK, GCKR, G6PC2, MTNR1B, DGKB-TMEM195, TCF7L2 and SLC30A8 were also confirmed in this study (140).

The MAGIC investigators also performed a meta-analysis looking for SNPs associated with HbA1C as a marker of glycaemic control, since the estimated heritability of HbA1C is relatively high (47-59%, (141)). They discovered 6 new loci associated with HbA1C including SNPs within FN3K, HFE, TMPRSS6, ANK1, SPTA1 and ATP11A/TUBGCP3. They also confirmed the association between previously identified genetic variants and HbA1C, these SNPs lie within the HK1, MTNR1B, GCK and G6PC2/ABCB11 genes (142).

The studies performed by the MAGIC investigators provide robust evidence that glycaemic control has a partial genetic basis. However, the potential clinical application this yields is unclear. It is possible that knowledge of these SNPs may facilitate identification of individuals at highest risk of

developing T2DM and allow targeted preventative measure. However, no studies to date have looked at this hypothesis or ventured any others regarding the clinical application of these findings.

Chapter 2: Research questions, Hypothesis and Objectives

2.1 Research Questions

In view of the findings discussed in Chapter One, four research questions were posed.

Firstly, is glycaemic control important in the development of diabetic cardiomyopathy? Is glycaemic control truly an independent risk factor for the development of LVH and HF rather than promoting diabetic cardiomyopathy indirectly through other pathological process such as CAD?

Secondly, is overly strict glycaemic control deleterious, as suggested by the ACCORD trial? More specifically, does overly strict glycaemic control promote the development of HF and LVH in T2DM? If so, what is the optimum range for glycaemic control with respect to HF and LVH development?

Thirdly, does the intra-individual level of variation in glycaemic control influence the development of HF in T2DM? If so, does this occur independently of overall average blood glucose levels?

Fourthly, is there a partial genetic basis for the two major phenotypes within diabetic cardiomyopathy, i.e. LVH and HF? Does this relate to the role of glycaemic control?

2.2 Hypotheses:

1. Both hyperglycaemia and hypoglycaemia may be associated with the development of LVH and HF in patients with T2DM
2. Greater intra-individual variation in blood glucose levels may increase the risk of HF development in T2DM
3. There is a partial genetic basis to T2DM, LVH and HF
4. The genetics of these pathologies are inter-twined

2.3 Objectives

To test these hypotheses, the extensive bioresources of the University of Dundee with its well-established data linkage methods were used to address the following objectives:

1. To assess how hyperglycaemia, hypoglycaemia and intra-individual blood glucose variation may be associated with heart failure development using longitudinal HbA1c measures to assess glycaemic control and hospital admission data, prescribing data and echocardiographic data to identify individuals with T2DM who developed LVH or/and HF during the study period
2. To investigate whether single nucleotide polymorphisms (SNPs) associated with LVH in previous genome-wide association studies were associated with LVH in the population of Tayside, Scotland with T2DM
3. To test the whether single nucleotide polymorphisms previously associated with glycaemic control were also associated with the development of HF independently of glycaemic control

Chapter 3: Methods

The methods of data analysis employed in this thesis are based on linkage of established clinical data sets.

3.1 Data collection: DARTS and Go-DARTS

The Health Informatics Centre, working in partnership with the University of Dundee and NHS Tayside, holds and maintains clinical data collected from the Tayside region of Scotland, which has a population of approximately 400 000. These data are anonymised in accordance with the Standard Operating Procedures approved by the Caldicott guardians. All individuals resident in Scotland and registered with a medical practitioner are assigned a Community Health Index number (CHI number). This is a person-specific, unique identifier consisting of 10 digits where the first 6 digits are the patient's date of birth. Every person with a CHI number appears in the Community Health Master Patient Index, held by the Tayside Health Board. This file contains address, post code, general practitioner, death and date of death for all persons with a CHI number, enabling basic demographic analysis of the Tayside population.

All healthcare activity in the region for the past 2 decades has been recorded using the CHI number, allowing data linkage with a high degree of reproducibility and accuracy. This has facilitated the creation of sophisticated regional health informatics systems, such as the Diabetes Audit and Research in Tayside Scotland (DARTS) clinical information system (143).

DARTS was established to facilitate diabetes research within Tayside using electronic record linkage. It was a joint initiative of the Department of Medicine and Medicines Monitoring Unit (MEMO), University of Dundee, the diabetes department within NHS Tayside and 8 general practitioners within NHS Tayside. Individuals with diabetes were identified through electronic record linkage of clinical data, a technique validated manually by consulting hospital written records, biochemistry data and general practice records for all patients listed with 8 randomly selected general practices

(GPs) in Tayside. This technique was shown to be more sensitive than identifying patients with diabetes through using GP registries alone (144).

The datasets comprising the DARTS clinical informatics systems are continually assimilated from multiple resources including the Community Health Master Patient Index, Scottish Morbidity Records (SMR), data from the General Registrar's Office (GRO), laboratory assays, data collected from diabetes clinics and regional prescribing data. Details of each of these databases are given below.

The Scottish Morbidity Record

The Scottish Morbidity Records (SMR) contain data from ICD (International Classification of Disease) coding representing discharge documentation following all acute hospital admissions in Tayside and Fife. The database consists of one entry per admission that includes date of admission, one principal diagnostic field and five additional diagnostic fields. Admissions for hospital procedures are also included with one principal procedure field and eight additional procedural fields. The hospital admissions are classified according to the International Classification of Disease (ICD) 9th and 10th versions. Procedures were classified according to the Office of Population, Censuses and Surveys Classification of Surgical Operations and Procedures, 3rd and 4th revisions.

General Registrar's Office Database

Data from the General Registrar's Office (GRO) provides mortality data and contains the date and cause of death for all deaths occurring in Tayside from 1989. The cause of death is coded according to the ICD classification systems referred to above. The database includes a principal cause of death field and up to 10 additional contributory causes of death fields in order to capture all data recorded on the death certificates.

Laboratory data

Records of all results from laboratory tests performed within NHS Tayside since 1992 are held in the regional biochemistry, haematology and microbiology databases. The use of results from biochemistry assays to identify those with diabetes based on oral glucose tolerance test and random plasma glucose results was validated in DARTS. Biochemistry results were available from 1989 onwards (144). The biochemistry database contains HbA1C values for DARTS participants, taken in view of the guidelines and recommendations referred to in Chapter One.

Dispensed prescription database

This database was originally established by MEMO, University of Dundee, in 1993 and is now maintained by HIC. It captures all prescriptions dispensed in the community within the Tayside region in a person-specific manner. Prescriptions dispensed between 1993 and 2004 were recorded as scanned paper prescriptions analysed with purpose written software. Since late 2004, data regarding all prescriptions were obtained in electronic format from the Practitioner Services Division (PSD).

Prescriptions within this database are linked to the individual's CHI number and include the generic name of the drug, date of prescription, amount dispensed, dosing instructions and drug codes as described in the British National Formulary (BNF).

SCI-DC

Since 2000, a nationwide clinical information system; the Scottish Care Information-Diabetes Collaboration (SCI-DC) database, has captured registration of patients with diabetes. Registration in the database occurs automatically when a patient is assigned a 'Read Code' for diabetes in a primary or secondary care electronic database. The SCI-DC database contains information on almost all patients with a diagnosis of diabetes resident in Tayside, Scotland. Clinical information is collected according to the national clinical dataset for the care of diabetic patients in Scotland and covers age, gender, diabetes type, date of diagnosis, blood pressure, height and weight among other clinical

parameters. The SCI-DC database has been linked to the clinical datasets described above in previous epidemiological studies within the Tayside population (144–146).

HEARTS

The HEARTS database is a web-based system allowing safe, secure access to clinical data from a central location. Secondary care investigation results (e.g. results from biochemistry assays performed in Tayside) are accessible through HEARTS. These data are linked to information held locally by primary care systems, such as GPASS, via person-specific CHI number. These data include height, weight and blood pressure measures, which were used in this project as detailed below.

Go-DARTS

Since 1998, an increasing number of patients with T2DM in the region have been approached to provide a blood sample for genetic studies and have given written informed consent for their data to be linked anonymously to data in DARTS and associated datasets for clinical research. These datasets are 'live' and continually updated. Record linkage via the CHI number has provided the means to electronically link this genetic data to the datasets described above. Each individual is assigned a unique system code number, the 'prochi', as part of the electronic linkage procedure, allowing them to maintain anonymity throughout. Many of these individuals were recruited under the auspices of the Wellcome Trust UK Type 2 Diabetes Case Control Consortium and contributed significantly to the replication phase of the WTCCC-GWA studies for T2DM (147). All Go-DARTS participants were seen by a research nurse at recruitment and baseline values for clinical parameters such as blood pressure, height and weight were recorded.

Data from this large group of patients with T2DM together with the varied longitudinal clinical datasets constitutes the Genetics of DARTS (Go-DARTS) study. Rigorous compliance with NHS data protection and encryption standards was maintained at all times. The Go-DARTS study was

approved by the local research ethics committee and has been described in several published articles (148–150).

3.2 The Tayside Echocardiography Database

The Tayside echocardiography database, maintained by the Department of Cardiology, contains data from all clinically requested echocardiograms performed in Ninewells Hospital, NHS Tayside from September 1993 and a small number of scans performed in Perth Royal Infirmary, NHS Tayside, during the same period. The database holds reports for a total of 10 387 echocardiograms performed on a total of 5 329 Go-DARTS participants. Out of 10 387, 9991 scans were performed in Ninewells Hospital, Dundee and 396 were performed at Perth Royal Infirmary. All echocardiograms were reported by echocardiographers with full accreditation of the British Society of Echocardiography.

The Tayside echocardiography database contains data for a total of 86 different variables including those pertaining to the identity of the individual (anonymised for data protection but maintaining data linkage), location and date performed. Both numeric values and free text data are included. The dataset is explicit for left ventricular size and function, including regional wall motion abnormalities, cardiac dimensions, valvular appearance, presence and extent of valvular stenosis and regurgitation (quantified according to British Society of Echocardiography recommendations), parameters indicating the level of diastolic function, presence of pericardial effusion with appropriate quantification and any additional comments made by the sonographer. The structured numerical fields were processed to ensure that any clearly erroneous, non-physiological measurements were removed prior to analysis.

As described above, data within the Tayside Echocardiography database is anonymised in a manner that still allows its contents to be linked to the datasets used within Go-DARTS. Permission from the Caldicott Guardian was obtained to allow the echocardiography database to be linked to the above

data sets for the purpose of this study. The echocardiography database was previously linked to the health informatics system at HIC to identify structural heart disease (151–153).

3.3 Record Linkage

The person-specific identifier referred to above, the CHI number, was converted to an anonymous, 10 character 'prochi' for all individuals in Go-DARTS. This approach minimized the risk of specific individuals being identified through the datasets, helping to maintain patient confidentiality.

The CHI number was also converted to a 'prochi' for all persons whose data was held within the Tayside Echocardiography database, allowing individuals to maintain anonymity. The Tayside Echocardiography Database may be linked to the Go-DARTS databases using data providing a link between the 2 projects. Permission for such linkage is sought from the Caldicott Guardian and is granted on a study-specific basis, as was the case for this study.

Linkage of the above described datasets can be described in diagram form as below:

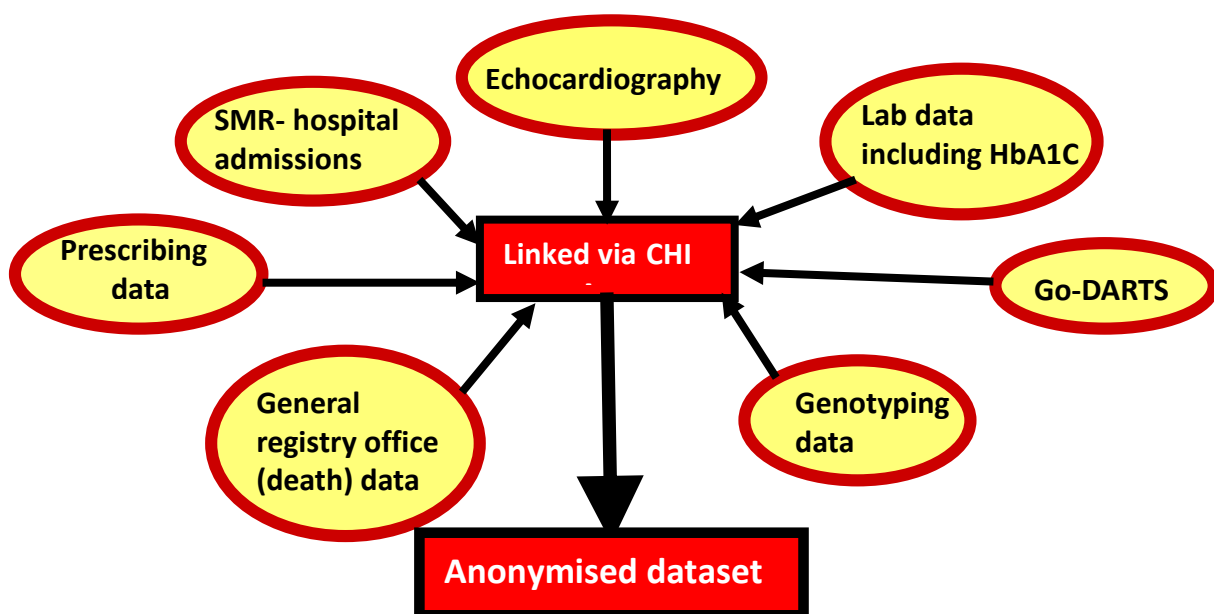


Figure 3.1 showing data-linkage procedure.

3.4 Heart failure phenotype

Individuals with T2DM who developed HF following enrolment in the Go-DARTS study were identified and extracted for a nested case-control study, longitudinal cohort study and genetics analysis. The same phenotype definition was used for all of these analyses. Individuals were regarded as having HF if they fulfilled at least one of the following two criteria:

- 1) Echocardiographic evidence of left ventricular systolic impairment and receipt of a loop diuretic prescription
- 2) Admission to hospital with HF and receipt of a loop diuretic prescription

Patients with T2DM were identified from the demographic database according to data within the 'diabetes type' field. The accuracy of the data relating to diabetes type has been well-validated in previous studies (143). Linking the SMR data and dispensed prescribing data to the Tayside Echocardiography database allowed identification of individuals with T2DM who met the above criteria for HF. Patients who were never prescribed a loop diuretic were not classed as HF cases as it was deemed that any individuals with symptomatic HF would have received as a loop diuretic at some point. This may have excluded some individuals with post-MI left ventricular dysfunction that either recovered so rapidly that out-patient loop diuretic was not required or was so well compensated that loop diuretics were not needed.

3.4.1 Identification of individuals with HF using echocardiographic data

Individuals with echocardiographic evidence of left ventricular systolic impairment were identified according to an algorithm generated to describe LV function in numeric form. In order to generate this algorithm, the free text conclusion and description of left ventricular systolic function (LVSF) were identified and processed. Code was developed to parse the free text, and to determine the sonographer's impression of left ventricular systolic function according to the standard terms described below. The code was developed in an iterative manner, initially using a small cohort of manually reviewed reports, until further improvement was not possible. The code was run through

the entire dataset and further development sought to reduce the number of un-interpreted reports. The code was modified to process the most commonly occurring typographical errors.

Every individual was assigned a number ranging from 1-6 or 9 to represent their level of LV systolic function where '1' describes normal LV function, '2' mildly impaired, '3' mild-moderate impairment, '4' moderate impairment, '5' moderate-severe impairment and '6' refers to severe impairment in LV systolic function. Individuals were assigned a '9' to reflect systolic function if their LV function could not be assessed according to the algorithm. For example, details of LV function may not be provided when individuals undergo focused imaging to assess a particular condition, these individuals would be among those assigned a '9'.

This algorithm was validated in 3 different ways. A random selection of 1000 reports were manually reviewed by an independent investigator and the correlation between the manual and code based interpretation was examined. Secondly, since echocardiographic images are infrequently of sufficient quality to allow accurate quantitative assessment of LV function and subjective qualitative assessment is more commonly used, the inter-observer variability was determined through identifying a random selection of 70 scans, with varying degrees of LV impairment. These scans were subsequently blindly re-reported by BSE accredited echocardiographers and Kappa analysis showed strong agreement between the values generated by the algorithm and re-reporting.

Lastly, a random sample of 105 individuals with LV systolic impairment on echocardiography who were also prescribed a loop diuretic were identified and their case notes were reviewed to identify whether they had a documented clinical diagnosis of HF. This allowed quantitation of the methodological reliability in identifying HF cases.

Data for all individuals with evidence of LV systolic dysfunction were linked to the dispensed prescribing data for the Go-DARTS study. Individuals who received a loop diuretic prescription were identified according to the BNF code 2.2.2. Thus, patients with T2DM, echocardiographic evidence of LV systolic impairment and in receipt of loop diuretics were extracted.

3.4.2 Identification of HF cases through SMR01 data

All individuals admitted to hospital due to HF were identified by the ICD codes for HF: ICD 9: 428, ICD10: I50. Individuals were regarded as having a hospital admission with HF if any of the 6 diagnostic fields relating to their admission contained the above ICD codes. These individuals were extracted and their data were linked to prescribing data to determine whether they had received a loop diuretic. Individuals in receipt of loop diuretics were identified using the BNF code 2.2.2.

3.4.3 Identification of incident HF cases

All patients with T2DM and HF according to echocardiographic and prescribing evidence were merged with those with a diagnosis of HF based on SMR01 and prescribing data. Each individual was assigned a date of HF diagnosis. Date of HF diagnosis was taken as either the date of the earliest echocardiogram showing left ventricular systolic impairment or the date of the earliest admission to hospital for HF. If the patient had both an echocardiogram showing left ventricular systolic impairment and an admission to hospital with HF the earlier of the 2 dates was taken to be the date of HF diagnosis. Anyone diagnosed with HF prior to enrolment in Go-DARTS was excluded from further analyses.

3.4.4 Identification of non-HF control subjects

Non-HF controls were identified for the nested case-control study. Individuals with T2DM were extracted from the demographic database as described above. Linkage to the echocardiography data, SMR01 data and dispensed prescribing data allowed identification of type 2 diabetics with no evidence of HF.

Non-HF controls had never had an echocardiogram showing evidence of LV systolic impairment. This included individuals who had undergone echocardiography and had their overall LV systolic function graded as normal (i.e. given a value of '1' according to the algorithm) and those individuals with T2DM who had never undergone echocardiography. Non-HF controls had also never had a

hospital admission with HF. Anyone with a discharge code for HF in any of the diagnostic fields was identified and excluded. Data for these individuals was linked to the dispensed prescribing data and anyone receiving a prescription with a BNF code for loop diuretics was excluded from the non-HF control group.

HF cases were matched to non-HF controls using the greedy matching process in SAS 9.2 (154). HF cases and non-HF controls were matched for age at diabetes diagnosis within 2 years, diabetes duration within 2 years and gender. Matched cases and controls used in the conditional logistic regression model are discussed below.

3.5 Phenotype definition LVH and case-control identification

Individuals within the Go-DARTS cohort who had T2DM were identified and extracted for a nested case-control analysis using a conditional logistic regression model to determine whether glycaemic control was associated with LVH development and to investigate whether genes previously associated with LVH were also associated with LVH in our cohort of patients with T2DM. Their data were linked to the echocardiography database as for the HF nested case-control study.

3.5.1 LVH case definition

Two-dimensional LV measurements taken in diastole for the interventricular septum (IVS), left ventricular posterior wall (LVPW) and left ventricular internal diameter (LVIDD) were used to identify individuals with LVH according to ASE criteria (88,155). LV measurements were used to calculate LV mass according to the formula:

$$\text{LV mass} = 0.8 * (1.04 * ((\text{LVIDD} + \text{LVPW} + \text{IVS})^3 - (\text{LVIDD})^3)) + 0.6$$

This formula is widely used within clinical and research fields and was derived and validated by Devereux *et al* (155,156). LV mass was separately indexed to height in metres to the power 2.7 and to body surface area (BSA). BSA was calculated using the formula below:

$$\text{BSA} = ((\text{weight})^{0.425} * (\text{height})^{0.725}) * 0.007184 \quad (157)$$

Individuals were classed as having LVH if their LV mass was outside the normal range when indexed to either height or BSA according to ASE recommendations (88). Participants with LV wall thickness exceeding the normal range according to direct 2D measures were also classed as cases of LVH. Additionally, individuals were regarded as having LVH if relative wall thickness (RWT) was increased using the formula:

$$\text{RWT} = (2 \times \text{LVPW}) / \text{LVIDD} \quad (158)$$

In summary, our LVH phenotype was defined according to ASE recommendations (88) and was comprised of individuals with T2DM and LVH according to one of the following criteria:

- 1) Raised LV mass
- 2) Increased LV wall thickness on direct 2D measurement
- 3) Increased RWT

Since significant aortic stenosis promotes LVH through increased left ventricular haemodynamic load, all patients with aortic stenosis of greater than mild severity were excluded from further analysis to prevent confounding.

3.5.2 Identification of control subjects

Patients with T2DM participating in Go-DARTS were classed as non-LVH controls if they had never had an echocardiogram nor received a loop diuretic. Data linkage with the dispensed prescribing data allowed identification of patients who had received a loop diuretic. Patients with prescriptions issued for BNF code 2.2.2 were extracted. Controls from this pool of individuals were matched to cases for gender, age at diabetes diagnosis and duration of diabetes within 2 years.

3.6 Calculation of weighted mean HbA1C

Single HbA1C measures underestimate the importance of glycaemic control. Calculation of the mean of serial HbA1C measures is a better predictor of diabetic complications than single HbA1C measures (159,160). In view of this, weighted mean HbA1C was calculated to investigate the

association between glycaemic control and HF development and glycaemic control and LVH development for each individual.

The number of days between 2 successive measures was multiplied by the HbA1C value for the first of these measures. This process was repeated for each HbA1C measure according to the formula below:

$$D_{x(1,2,\dots,\text{end})} = \text{HbA1C}_{x(1,2,\dots,\text{end})} * (dt_{(x+1)} - dt_x)$$

where the HbA1C value is in per cent, 'dt' represents the date the specimen was taken and 'x' refers to the specimen number, i.e. first, second, etc.

In order to calculate weighted mean HbA1C, the sum of the above values was taken and then divided by the number of days between the initial HbA1C sample and the last, i.e. the duration of time over which the HbA1C values were taken. This process is summarised by the formula below:

$$\text{Weighted mean HbA1C} = \sum D / (\text{last_date} - dt_1)$$

where 'last_dt' represents the date of the last HbA1C measure before development of HF and LVH for HF and LVH cases. For those who did not develop HF, HbA1C duration was calculated differently for the nested case control and the longitudinal cohort studies. For the nested case-control studies, the HbA1C duration was taken as the time between first HbA1C measure and last measure before HF development, or first echocardiogram showing LVH, for the respective case for matched controls. For the longitudinal cohort studies HbA1C duration was taken as time from the first HbA1C measure to the end of the study period. Both calculations were performed using the array statement in SAS 9.2 for Windows.

Weighted mean HbA1C was calculated as described above for each individual in the study and this was used to represent long-term glycaemic control in all subsequent logistic regression and proportional hazard regression models.

3.7 Analysis of the relevance of anti-hyperglycaemic medications

The prescribing data were linked to the demographic data to extract information regarding anti-hyperglycaemic medication use for all study participants. These data were handled differently for the case-control and longitudinal cohort studies.

3.7.1 Analysis of anti-hyperglycaemic medications: nested case-control studies

All prescriptions issued within the 3 months preceding HF development for HF cases were extracted. For control subjects, all prescriptions issued within the 3 months preceding HF development for their respective cases following the matching process were extracted. A similar process was performed for LVH cases. Date of first echocardiogram showing LVH was taken as index date for LVH cases and for their respective controls.

All prescriptions for anti-hyperglycaemic medications dispensed within this period were then identified. Sulphonylurea prescriptions were identified by the BNF code 6.1.2.1, metformin prescriptions were identified by the BNF code 6.1.2.2 and insulin prescriptions were identified by the BNF codes 6.1.1.1, 6.1.1.2 and 6.1.1.3. Prescriptions for thiazolidinediones (TZDs) were identified using their approved names: 'pioglitazone' and 'rosiglitazone'.

Every individual was then assigned to one of 5 categories of treatment: insulin therapy, sulphonylurea therapy, TZDs, metformin or diet-control. Patients were categorised as taking insulin, sulphonylureas, TZDs, metformin or diet-control. Anyone taking insulin was assigned to the insulin category, anyone not taking insulin but taking sulphonylureas was assigned to the sulphonylureas category, anyone taking neither insulin nor sulphonylureas but taking TZDs was assigned to the TZDs category and anyone taking metformin only was assigned to the metformin category. Patients with no prescriptions for anti-hyperglycaemic medication during the period in question were classed as receiving diet-control.

3.7.2 Analysis of anti-hyperglycaemic medications: HF longitudinal cohort study

Anti-hyperglycaemic medications prescribed at entry into the Go-DARTS study were identified and these baseline data were used in the proportional hazard regression model for the HF cohort study. Anti-hyperglycaemic medication prescriptions were identified using the BNF codes given above and treatment was categorised as insulin therapy, sulphonylureas, other oral therapy or diet-control in the same way as for the nested case-control studies.

3.8 Analysis of the role of CAD in the development of HF and LVH

All study participants were subdivided into those who had CAD and those who did not based on hospital admission with discharge codes relating to ischaemic heart disease. These included the following codes ICD 9: 410, 411, 412, 413 or 414 and ICD10: I20, I21, I22, I23, I24 or I25.

3.9 Identification of covariates through Students' t test and Chi-squared test of association

Multiple covariates have been shown to influence the development of HF and LVH. These include age, gender, blood pressure (BP), body mass index (BMI), renal function, presence and duration of diabetes and CAD (161). Anti-hyperglycaemic medications including insulin, sulphonylureas, metformin and thiazolidinediones have been associated with HF. In view of this, the Students' t test and Chi-squared test of association were used to identify variables associated with HF and LVH in our population of individuals with T2DM.

Students' t test was performed to identify the continuous variables associated with HF and LVH. Age at enrolment into Go-DARTS and age at diabetes diagnosis were calculated using the data within the demography file. The clinical datasets described above, including the SCI-DC, HEARTS and WTCCC data, were linked to provide values for BMI, systolic and diastolic BP and creatinine.

BMI was calculated using the standard formula, as given below:

$$\text{BMI} = \text{weight (kg)} / (\text{height (m)})^2$$

Most cohort members had multiple measurements for weight, systolic and diastolic BP and creatinine. Where multiple values were available, the value closest to the date of enrolment in the

Go-DARTS study was taken, as this most closely represented baseline status. Students' t test was then performed to identify which of the above variables were associated with HF and LVH, thus identifying the covariates to be taken forward for use in logistic regression modelling.

Individuals with CAD were identified according to the ICD 9 and ICD 10 codes described above.

Individuals with CAD were assigned a '1' for the CAD variable and those without CAD according to the above criteria were assigned a value of '0'. Medication use was categorised as described above.

Chi-squared test of association was then used to determine whether CAD status and gender were associated with HF and LVH and whether prescription of specific anti-hyperglycaemic medications was linked to HF.

3.10 Identification of independent covariates through logistic regression analysis

Conditional logistic regression analysis was performed to confirm whether covariates associated with HF and LVH were independent predictors of HF and LVH respectively when all other significant covariates were taken into account.

3.10.1 Conditional logistic regression analysis: glycaemic control and HF

Conditional logistic regression analysis was performed in which HF was modelled as a binary trait.

All clinical features shown to be significantly associated with HF development according to the above analyses were included in the model as covariates. Hence, covariates included CAD status, SBP, BMI and creatinine category. Values for creatinine measurements were categorised due to the broad range of values involved: values less than 80 umol were included in the same category, values greater than 80 umol were categorised in 10 umol increments up to 200 umol. Measures of creatinine exceeding 200 umol were placed in a final category. Creatinine categories were assigned numeric values starting with '1' for those with baseline creatinine <80 umol, '2' if baseline creatinine was between 80 and 90 umol with all subsequent categories assigned ascending numbers in this manner. Systolic BP and BMI were not categorised due to the narrower range of values.

The logistic regression model was stratified so that each HF case was compared specifically to the control individual he/she had been matched with. Covariates within this model were regarded as significant independent predictors of HF if their corresponding p-value was <0.05 . These covariates were taken forward for use in the longitudinal cohort study proportional hazard regression analysis. Between 6% and 10% weighted mean HbA1C was stratified in 1% increments to assess how specific levels of glycaemic control were associated with HF. All individuals with weighted mean HbA1C $<6\%$ and $\geq 10\%$ were classified according to these categories. Weighted mean HbA1C ≥ 6 to 7% was taken as the reference range for this analysis and all the regression analyses described below.

3.10.2 Conditional logistic regression analysis: glycaemic control and LVH

Conditional logistic regression analysis was used to model LVH as a binary trait to investigate the association between weighted mean HbA1C and LVH. Cases and controls were defined according to the above criteria. Independent variables associated with LVH according to Students' t test and the Chi-squared test of association were included in the model as covariates. Hence, covariates included age, gender, weighted mean HbA1C, SBP, DBP, BMI, creatinine category and CAD status. Weighted mean HbA1C categorised as above.

3.11 Longitudinal cohort study

3.11.1 Proportional hazard regression analysis: optimum range for glycaemic control with respect to HF

All patients with T2DM enrolled in Go-DARTS during the study period were included in the cohort for proportional hazard regression analysis. Weighted mean HbA1C was calculated using all available values until HF diagnosis for cases and all available values until the end of the study period for all other members of the cohort. Start date was taken as date of enrolment into the Go-DARTS study and the study end point was the date of the last diagnosis of HF according to the data collected, i.e. 27th August 2011. Patients who did not develop HF during the study period were censored.

Proportional hazard regression analysis was used to determine the hazard ratio of developing HF for each level of weighted mean HbA1C according to the categories <6%, ≥6 to 7%, ≥7 to 8%, ≥8 to 9%, ≥9 to 10% and ≥10%.

All factors associated with HF according to the conditional logistic regression model described above contributed to the proportional hazard regression model. These variables included BMI, SBP, creatinine category, age, gender, diabetes duration and CAD. Values for BMI, creatinine, age and diabetes duration were taken at baseline. Existence of CAD was modelled in a time-dependent manner such that individuals who developed CAD according to the above definition prior to the development of HF were classed as CAD cases and anyone developing CAD after they were diagnosed with HF was not classed as a CAD case for the purpose of this model. Proportional hazard regression modelling was performed with and without the inclusion of CAD in the list of covariates to assess whether the relationship between weighted mean HbA1C and the development of HF was dependent on CAD by comparing hazard ratios across the 2 models.

As discussed above, anti-hyperglycaemic agents may influence HF development in T2DM. To assess the impact of anti-hyperglycaemic medications, all members of the cohort were assigned to one of 4 treatment categories according to drug prescription at study enrolment. Drug therapy was determined using BNF codes as described above. Patients were categorised as taking insulin, sulphonylureas, other oral therapy or diet-control. Treatment was then added into the proportional hazard regression model using individuals categorised as diet-controlled as the reference group.

The Chi-squared test of association was used to compare frequencies of anti-hyperglycaemic medications prescribed to HF cases versus non-HF cases within 3 separate groups: patients with weighted mean HbA1C within the range (≥6 to 8%), patients with relative hypoglycaemia (weighted mean HbA1C <6) and patients with relative hyperglycaemic (weighted mean HbA1C ≥8%). Eight per cent was used as the upper limit of normal for weighted mean HbA1C as the above analyses showed increased risk of HF development above this level, as described below. Patients taking either insulin

or sulphonylurea therapy were grouped together as these agents are secretagogues and known to promote hypoglycaemia. Patients who were not taking insulin or sulphonylureas who were taking either metformin or TZDs were grouped together as these are insulin-sensitizers.

Calculation of HbA1c variability

To investigate the effects of intra-individual variations of HbA1c on incident HF we calculated intra-individual mean (HbA1c-MEAN) and standard deviation (HbA1c-SD), respectively. HbA1c values obtained preceding recruitment, at the enrolment and until the last recorded value of HbA1c were used. The inter-individual difference in the number of HbA1c assessments was adjusted according to the formula: $\text{adj-HbA1c-SD} = \text{SD}/\sqrt{n/(n-1)}$ as previously described (162). HbA1C variability was then included as in the proportional hazard regression analysis.

All analyses were carried out using SAS 9.2 for Windows and the R software (165).

3.11.2 Proportional hazard regression analysis: optimum range for glycaemic control with respect to LVH

All patients with T2DM enrolled in Go-DARTS during the study period were included in the cohort for proportional hazard regression analysis. Weighted mean HbA1C was calculated using all available values until first echocardiogram showing LVH for cases and all available values until the end of the study period for all other members of the cohort. Start date was taken as date of enrolment into the Go-DARTS study and the study end point was the date of the last diagnosis LVH according to the data collected, i.e. 8th September 2011. Patients who did not develop LVH during the study period were censored. Proportional hazard regression analysis was used to determine the hazard ratio of developing LVH for each level of weighted mean HbA1C according to the categories <6%, >=6 to 7%, >=7 to 8%, >=8 to 9%, >=9 to 10% and >=10%.

All factors associated with LVH according to the conditional logistic regression model described above contributed to the proportional hazard regression model. These variables included BMI, SBP, creatinine category, age, gender, diabetes duration and CAD. Values for BMI, creatinine, age and diabetes duration were taken at baseline. Existence of CAD was modelled in a time-dependent manner such that individuals who developed CAD according to the above definition prior to the development of LVH were classed as CAD cases and anyone developing CAD after they were diagnosed with LVH was not classed as a CAD case for the purpose of this model. Proportional hazard regression modelling was performed with and without the inclusion of CAD in the list of covariates to assess whether the relationship between weighted mean HbA1C and the development of LVH was dependent on CAD by comparing hazard ratios across the 2 models.

To assess the impact of anti-hyperglycaemic medications, all members of the cohort were assigned to one of 5 treatment categories according to drug prescription at study enrolment. Drug therapy was determined using BNF codes as described above. Patients were categorised as taking insulin, sulphonylureas, TZDs, metformin or diet-control. Categories were assigned as described above. Prescriptions within 3 months of the index date were considered. Patients with no prescriptions for anti-hyperglycaemic medication during this period were classed as receiving diet-control.

Treatment was then added into the proportional hazard regression model using individuals categorised as diet-controlled as the reference group. All analyses were carried out using SAS 9.2 for Windows and the R software (163).

3.12 Genotyping procedures for HF and LVH studies

Genotyping was performed on a proportion of Go-DARTS participants who had been prescribed statins for an unpublished study looking at statin response. The clinical data for the HF and LVH analyses described above were linked to the available genotyping data to investigate the potential genetic basis of HF and LVH.

3.12.1 High Density Array Genotyping Data

Blood samples were donated by Go-DARTS participants for genotyping as previously stated. The Genome-Wide Human SNP Array 6.0 was used. Specimens were processed at the Affymetrix service laboratory. All samples passing Affymetrix laboratory quality control were taken forward to call genotypes with using the Chiamo (164) software adapted for Affymetrix 6.0 SNP data. Direct typing of individual SNPs was performed using TaqMan allelic discrimination assays as supplied by Applied Biosystems (Carlsbad, CA) as “Assays on Demand”, or “Assays by Design”.

3.12.2 DNA preparation and genotyping

The genomic DNA quality was initially validated using the Sequenom iPLEX assay. DNA concentration was then assessed using the PicoGreen assay Invitrogen, all samples with DNA concentration above threshold were taken forward and tested by agarose gel electrophoresis. DNA not degraded by this process was used.

3.12.3 Sample Quality Control

Genotype data quality control was performed in a similar fashion to other Wellcome Trust Case Control Consortium 2 (WTCCC2) studies that were previously published (165–169).

Genotyping data were modelled as a mixture of 'normal' and 'outlier' individuals for: ancestry, missing data, heterozygosity and gender assignment. Each model was fitted using a Bayesian framework generating a posterior probability of that individual being an 'outlier'. Individuals with a posterior probability exceeding the 0.5145 threshold were regarded as 'outliers' and excluded from further analysis.

Genome-wide levels of allele sharing were compared to assess 'relatedness' and filter-out pairs who were identical by descent using PLINK (170).

3.12.4 Affymetrix 6.0 SNP genotyping array

Four thousand individuals with diabetes were genotyped on the Affymetrix 6.0 SNP genotyping array; which includes 1 000 000 SNPs. These individuals were specifically chosen for genotyping as part of a previous project based on the fact that they had received a dispensed prescription for statin treatment subsequent to their enrolment in the Go-DARTS study.

3.13 Manipulation of the genetic data including imputation

3.13.1 Imputation

High density SNP array data were stored using the Chiamo (164) and the PLINK binary PED (170) formats. The Chiamo format was used for analyses run in SNPTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snpctest/snpctest.html (171)) while the PLINK binary PED format was used for the analyses run in PLINK (170).

Genotype imputation was performed in two stages. The SHAPE-IT tool was used to estimate haplotypes from the study population (<http://www.shapeit.fr/> (172)) prior to comparing haplotypes to reference panel haplotypes using IMPUTEv2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html (173)), which compared the estimated haplotypes generated using the SHAPE-IT tool with up to 2 reference panels. This is based on the principle that certain SNPs will be found in haplotypes together.

3.13.2 Genetic associations and outcome: LVH

Genotyping association was determined using the SCORE TEST in SNPTEST (171) and the 'recode A' option in PLINK (170) to investigate whether published SNPs associated with LVH were also associated with LVH in this cohort with T2DM.

For directly typed SNPs in both SNPTEST and PLINK, an individual is assigned a value of '0' if they are homozygous for the non-effect allele, '1' if they are heterozygous and '2' if they are homozygous for the effect allele at each SNP locus. The data for each SNP can then be added to the logistic regression model in the same way as non-genetic variables.

Imputed SNPs are more complex to model and are estimated based on linkage disequilibrium with the directly typed SNPs as described above. Different SNPs are imputed with varying degrees of certainty; both the missing data likelihood score test in SNPTEST and the imputation functions in PLINK generate a probability that the genotype for that SNP is actually there, although non-observed. Imputed SNPs are dealt with differently in SNPTEST and PLINK. SNPTESTv2 (https://mathgen.stats.ox.ac.uk/genetics_software/snpctest/old/snpctest.html) uses a missing data likelihood score test. Different SNPs are imputed with varying degrees of probabilities of each genotype across individuals for a SNP and use all the available data in the association analysis. PLINK uses a threshold method such that if the probability of a specific genotype at a SNP locus exceeds 0.90 then that genotype is assumed to be there (170). This output can then be modelled by logistic regression as described for directly typed SNPs.

3.13.3 Meta-analysis

Meta-analysis was performed to assess the likelihood the above SNPs were associated with LVH when our data was combined with published data. The odds ratios calculated for the published SNPs were based on continuous data so our binary case-control model was adjusted to allow comparison with published data. Continuous data for LV thickness and LV mass were used as outcome measures. Individual LV mass values were based on calculations using the equation detailed above and values for LV thickness were taken as the sum of the IVS and LVPW. A linear regression model was used to determine whether published SNPs associated with LV mass were associated with continuous measures of LV mass in this study. Further linear regression modelling was undertaken to assess whether either of the published SNPs associated specifically with LV thickness were associated with continuous measures of LV thickness in this study. These results were then meta-analysed using the weighted z values calculated using the inverse variance-weighted z-score as described by Bakker et al (174) since the units used in the published GWAS differed from our own.

3.13.4 Calculation genotypic scores

Genotypic scores were calculated both for LVH and glycaemic control. SNPs were coded 0, 1 and 2 as described above. For the LVH gene score, effect alleles were defined as those associated with LVH development as published by Shah *et al* and Vasan *et al* (105,106). For the glycaemic control score, effect alleles were defined as those identified by the MAGIC consortium in the referenced articles that were shown to be associated with glycaemic control.

Three separate genotypic scores for LVH were calculated for every participant based on the number of effect alleles the participant had for each SNP associated with LVH. SNPs discovered by Vasan *et al* (105) were combined to produce gene score 1, which included rs17568359, rs7565161, rs7910620, rs2059238 and rs17132261. SNPs discovered by Shah *et al* (106) were combined to produce gene score 2, including rs6797133, rs2292462, rs2290893 and rs4966014. All the above SNPs discovered by both Vasan *et al* and Shah *et al* were combined to produce gene score 3. Gene scores were then categorized by quintiles and association with LVH was assessed by logistic regression analysis. Each SNP was weighted by the published per affect allele increase in LV dimensions using the SCORE option in PLINK. A 'SCORE' value is generated by PLINK by multiplying the number of reference alleles at that SNP locus by the published effect size at that SNP then adding these values across all reference SNPs for that individual. The association between LVH and the 3 genotypic score was then assessed by merging the genotypic score data with the clinical data previously described and adding the 'SCORE' variable into the logistic regression model. In view of the small sample size, the values for genotypic score were categorised according to quintiles; which replaced the 'SCORE' variable in the logistic regression model. Hence, 3 separate analyses were performed, each with 5 possible permutations of the gene score. The score for the analysis using gene score 3 was dependent on the scores for gene score 1 and 2.

3.13.5 Replicated SNPs associated with LVH: effect on outcome

Survival analysis and proportional hazard regression analysis were performed to assess the impact variation in the replicated SNPs had on outcome. The Lifetest Procedure was performed in SAS 9.2 plotting time to death, hospital admission due to cardiovascular illness and a composite end-point of either death or hospital admission due to cardiovascular illness. Proportional hazard regression analysis was then performed modelling each of the above outcomes including the number of effect alleles for each replicated SNP amongst the covariates.

3.13.6 Glycaemic control SNPs: Genetic associations and outcome

The 'recode A' option in PLINK was used to identify genotype with respect to all SNPs associated with glycaemic control as published by the MAGIC consortium (140,142). These included rs560887, rs10830963, rs4607517, rs2191349, rs780094, rs11708067, rs7944584, rs10885122, rs174550, rs11605924, rs11920090, rs7034200, rs340874, rs11071657, rs11558471, rs13266634 and rs4506565, associated with fasting glucose at genome-wide significance (140). SNPs associated with HbA1C at genome wide significance were also analysed (142). These included rs2779116, rs552976, rs1800562, rs1799884, rs6474359, rs4737009, rs16926246, rs1387153, rs7998202, rs1046896 and rs855791. All available genotypic data relating to the above SNPs was extracted for individuals classed as HF cases, non-HF cases, LVH-cases and non-LVH cases based on the definitions of HF and LVH given above. This genotypic data was then merged with clinical data and proportional hazard regression analysis was performed to determine whether these SNPs were associated with HF or LVH when known risk factors for both conditions were taken into account, as described in section 3.11.1.

Chapter 4: Results

A total of 8 673 participants in the Go-DARTS study had T2DM. These individuals formed the cohort from which individuals were extracted for all the above analyses.

4.1: LVH in T2DM

4.1.1 Frequencies of LVH cases and non-LVH controls

A total of 2141 out of the 8 673 individuals with T2DM had LVH according to echocardiography based on the definition described in section 3.5.1, representing 2141 out of 3510 individuals with T2DM (61%) in Go-DARTS who underwent echocardiography. One hundred and eighteen of these individuals had aortic stenosis of greater than mild severity. These individuals were excluded from further analysis to prevent confounding. The remaining cases of LVH included 1419 cases of LVH based on LV mass indexed to height, 1435 cases of LVH based on LV mass indexed to BSA, 1949 based on 2D LV measurements in diastole and 1361 based on RWT. There was significant overlap between the groups, i.e. many individuals met criteria for LVH based on more than one of the above definitions. Out of the 2023 individuals with LVH and T2DM, 1166 had prevalent LVH and 1139 LVH cases could be matched to non-LVH controls for age within 2 years, date of diabetes diagnosis within 2 years and gender. These figures are summarized in the flow chart below:

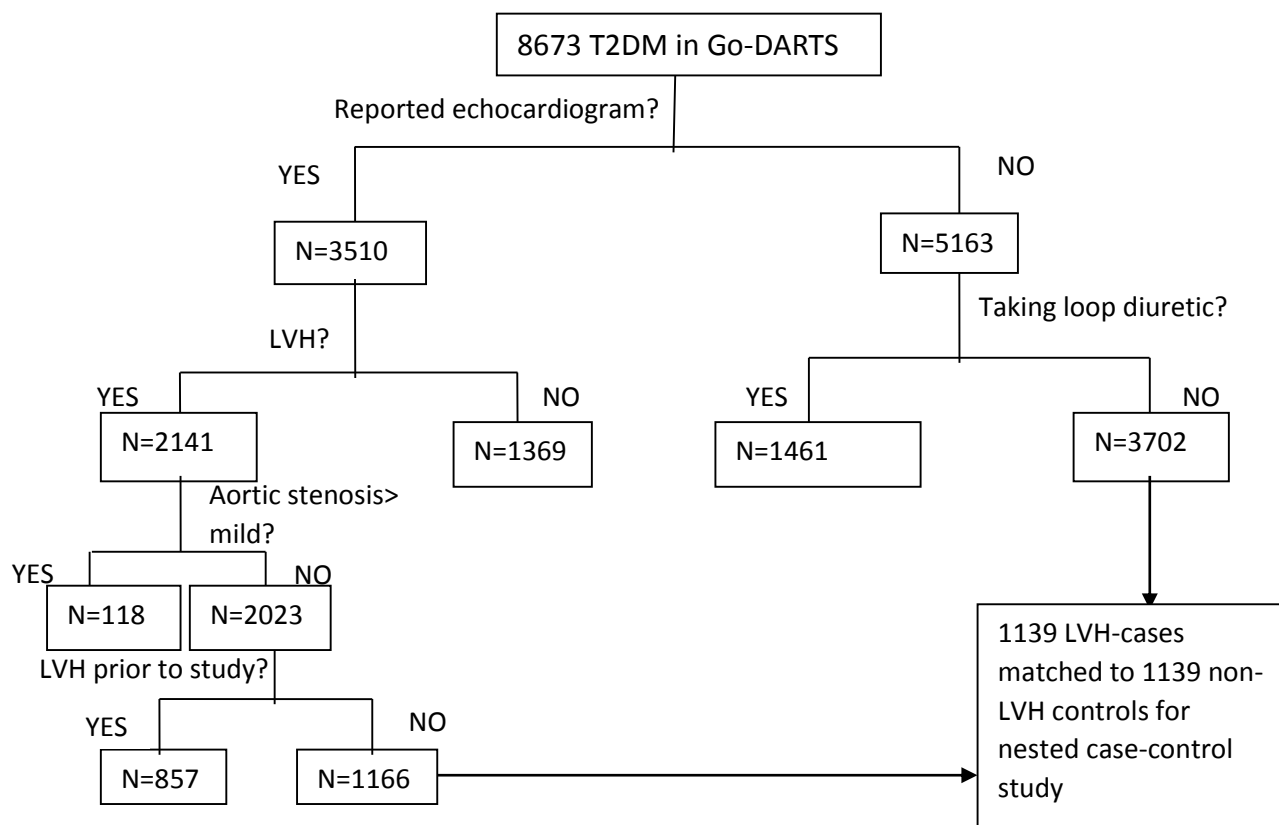


Figure 4.1.1: Identification of LVH-cases and non-LVH cases for the nested case-control study

4.1.2 Clinical characteristics: LVH cases and non-LVH cases

Clinical characteristics for all LVH cases and non-LVH cases are summarised in the table below.

Variable	LVH cases	Non-LVH cases	Significance, p=
N	1139	1139	NA
Number HbA1C measures (SD)	27.33 (13.94)	23.90 (12.01)	<0.01
Time period HbA1C measured, yrs (SD)	9.07 (3.96)	8.65 (4.15)	0.01
Age, yrs (SD) at diag	59.96 (10.70)	60.06 (10.65)	0.81
Number of males (%)	630 (55.31)	630 (55.31)	1.00
Duration T2DM, yrs (SD)	11.25 (6.42)	11.19 (6.39)	0.83
Frequency CAD (%)	598 (53.01)	196 (17.56)	<0.01
SBP, mmHg (SD)	138.20 (21.54)	140.47 (17.37)	<0.01
DBP, mmHg (SD)	72.40 (11.91)	76.38 (9.94)	<0.01
BMI, kg/m ² (SD)	31.06 (5.85)	29.06 (5.32)	<0.01
Height, m (SD)	1.67 (0.10)	1.67 (0.10)	0.10
Creatinine, umol/L (SD)	110.76 (62.92)	91.36 (21.70)	<0.01
Weighted mean HbA1C % (SD)	7.55 (1.36)	7.38 (1.39)	<0.01
Insulin at LVH diagnosis (%)	358 (31.43)	189 (16.59)	<0.01
SUs at LVH diagnosis (%)	363 (31.87)	391 (34.33)	0.21
Metformin at study enrolment (%)	425 (37.31)	475 (41.70)	0.03
TZDs at study enrolment (%)	112 (9.83)	102 (8.96)	0.47
Diet control at LVH diagnosis (%)	215 (18.88)	341 (29.94)	<0.01

Table 4.1.1: Clinical characteristics of LVH cases and non-LVH controls, nested case-control study

The matching process was deemed successful as there were equal numbers of males and females in each group and there was no significant difference in age at diabetes diagnosis or duration of diabetes between the 2 groups.

There was a significant association between development of LVH and increasing number of HbA1C measures, time period over which measures were taken, SBP, DBP, BMI, creatinine and weighted mean HbA1C. There was also a significant association between history of CAD, baseline insulin use and LVH. Metformin and diet-control appeared to be protective according to baseline association testing. These covariates were taken forward for use in the logistic regression model for the nested case-control study.

4.1.3 Independent risk factors for LVH development in T2DM: nested case-control study

Conditional logistic regression modelling showed CAD, BMI, creatinine and weighted mean HbA1C were significant independent predictors of LVH development. In this model, weighted mean HbA1C was categorised into 6 groups: <6%, ≥6 to 7%, ≥7 to 8%, ≥8 to 9%, ≥9 to 10% and greater than or equal to 10%. Weighted mean HbA1C ≥6 to 7% was taken as the reference range. Weighted mean HbA1C ≥8 to 9% and ≥9 to 10% were associated with increased risk of LVH development compared to the reference range (table 4.1.2).

Whilst BMI and creatinine remained independent predictors of LVH irrespective of CAD status, glycaemic control was less important and only weighted mean HbA1C ≥7 to 8% was associated with increased risk of LVH both in the group of individuals with CAD and the CAD-free group.

Table 4.1.2: Independent predictors of LVH in T2DM according to logistic regression modelling

Variable	OR	95% CI	P value
CAD	7.68	5.80-10.17	<0.01
SBP	1.00	0.99-1.00	0.17
BMI, kg/m ²	1.08	1.05-1.10	<0.01
Creatinine, umol/L	1.21	1.15-1.27	<0.01
M_HbA1C <6 %	1.23	0.81-1.85	0.33
M_HbA1C ≥6-7%	1.00	1.00-1.00	NA (ref)
M_HbA1C ≥7-8%	1.25	0.94-1.65	0.13
M_HbA1C ≥8-9%	1.60	1.14-2.26	<0.01
M_HbA1C ≥9-10%	1.64	1.02-2.66	0.04
M_HbA1C ≥10%	1.04	0.56-1.95	0.90

4.1.4 Proportional hazard regression analysis: optimum range for glycaemic control with respect to LVH

Proportional hazard regression analysis showed the hazard ratio of developing LVH was significantly increased with weighted mean HbA1C below 6% and above or equal to 8% as shown in Figure 4.1.2 and Table 4.1.3. Hazard ratio increased significantly with HbA1C greater than 8%. However, weighted mean HbA1C below 6% was associated with greater risk of HF development than weighted mean HbA1C ≥8 to 9% (HbA1C below 6% HR 2.0, 95% CI 1.6-2.4, $p=1.4 \times 10^{-9}$, HbA1C ≥8 to 9% HR 1.2, 95% CI 1.2-1.5, $p=0.02$).

Age, sex, duration of diabetes, history of CAD, BMI, creatinine category, SBP and DBP were all independent predictors of HF development in T2DM according to the proportional hazard regression model (Table 4.1.3).

Figure 4.1.2: Relationship between weighted mean glycosylated haemoglobin (HbA1C) and the development of LVH in type 2 diabetes, including age, duration of diabetes, gender, history of coronary artery disease, body mass index, creatinine, systolic and diastolic blood pressure as covariates

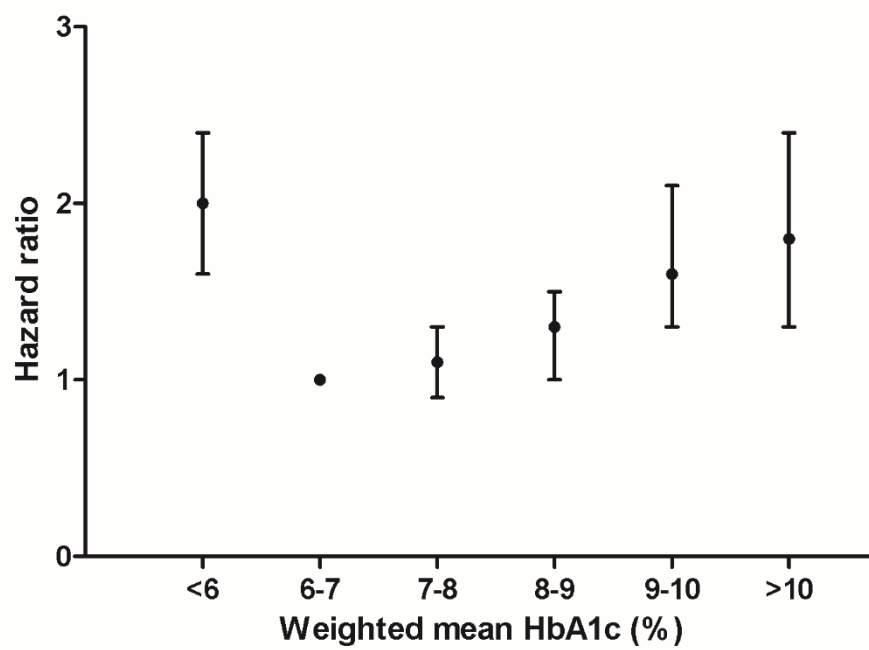


Table 4.1.3: Risk of LVH development associated with varying levels of glycaemic control

Variable	Hazard ratio	95% CI	P value
Age, years	1.04	1.03-1.05	6.05x10 ⁻³⁰
Gender	0.85	0.76-0.97	0.01
Duration diabetes, years	1.01	1.00-1.02	0.01
SBP, mmHg	1.01	1.00-1.01	1.83x10 ⁻⁴
DBP, mmHg	0.99	0.98-0.99	2.32x10 ⁻⁴
CAD	3.60	3.19-4.06	2.97x10 ⁻⁹⁷
BMI, kg/m ²	1.06	1.04-1.07	5.21x10 ⁻²³
Creatinine category, umol/L	1.08	1.06-1.11	4.57x10 ⁻¹⁰
Weighted mean HbA1C <6%	1.95	1.57-2.43	1.45x10 ⁻⁹
Weighted mean HbA1C ≥6-7%	1.00	1.00-1.00	NA (reference)
Weighted mean HbA1C ≥7-8%	1.10	0.94-1.28	0.23
Weighted mean HbA1C ≥8-9%	1.25	1.04-1.50	0.02
Weighted mean HbA1C ≥9-10%	1.64	1.29-2.09	5.97x10 ⁻⁵
Weighted mean HbA1C ≥10%	1.80	1.32-2.44	1.89x10 ⁻⁴

CI=confidence interval, P-value=probability value, HbA1C= glycosylated haemoglobin, yrs=years, T2DM=type 2 diabetes mellitus, CAD =coronary artery disease, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, SU=sulphonylurea, TZD=thiazolidinedione, ref=reference group

4.1.5 Role of CAD in the relationship between glycaemic control and incident LVH

Proportional hazard regression analysis was repeated excluding CAD from the list of covariates to investigate whether the relationship between LVH and glycaemic control was driven by an association between CAD and glycaemic control. There was a significant increase in the risk of developing LVH with weighted mean HbA1C <6% in both models (HR 1.95 with the inclusion of CAD, HR 2.14 when CAD was not taken into account). There was a significant increase in the risk of LVH development with all weighted mean HbA1C categories greater than or equal to 8% (HbA1C \geq 8 to 9% HR 1.4, 95% CI 1.2-1.7, HbA1C \geq 9 to 10% HR 1.8, 95% CI 1.4-2.3, HbA1C \geq 10% HR 2.3, 95% CI 1.7-3.1) whereas weighted mean HbA1C \geq 7 to 8% was not associated with significantly increased risk of LVH development in this CAD-free model (HR 1.1, 95% CI 0.9-1.3). These results are summarised in Table 4.7b. Overall, exclusion of CAD from the list of covariates did not make a difference to the pattern seen in the relationship between weighted mean HbA1C.

Table 4.1.4: Risk of LVH development associated with varying levels of glycaemic control, excluding CAD from the covariates

Variable	Hazard ratio	95% CI	P-value
Age, years	1.05	1.04-1.06	9.49x10 ⁻⁴²
Gender	0.91	0.81-1.03	0.14
Duration diabetes, years	1.02	1.02-1.03	2.61x10 ⁻⁴
SBP, mmHg	1.01	1.00-1.01	1.89x10 ⁻⁵
DBP, mmHg	0.98	0.98-0.99	1.56x10 ⁻⁸
BMI, kg/m ²	1.06	1.05-1.07	3.15x10 ⁻²⁹
Creatinine category, umol/L	1.12	1.09-1.14	6.47x10 ⁻¹⁸
Weighted mean HbA1C <6%	2.14	1.72-2.65	6.52x10 ⁻¹²
Weighted mean HbA1C ≥6-7%	1.00	1.00-1.00	NA (ref)
Weighted mean HbA1C ≥7-8%	1.09	0.93-1.27	0.28
Weighted mean HbA1C ≥8-9%	1.41	1.18-1.69	1.92x10 ⁻⁴
Weighted mean HbA1C ≥9-10%	1.81	1.42-2.31	1.57x10 ⁻⁶
Weighted mean HbA1C ≥10%	2.27	1.67-3.08	1.60x10 ⁻⁷

CI=confidence interval, P-value=probability value, LVH=left ventricular hypertrophy, CAD=coronary artery disease, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, HbA1C=glycosylated haemoglobin, CI=confidence interval, p-value= probability value occurring by chance.

4.1.6 Role of anti-hyperglycaemic agents in the relationship between glycaemic control and LVH development

Prescription of anti-hyperglycaemic medications also differed significantly in the LVH cases relative to non-LVH patients. Insulin prescribing rates were significantly greater in cases relative to non-LVH cases, metformin prescribing rates were significantly lower in LVH cases relative to non-LVH cases and fewer LVH cases were within the diet-control category compared to non-LVH cases. There was no significant difference in prescribing rates for sulphonylureas and TZDs in LVH cases compared to non-LVH cases (Table 4.1.1).

The proportional hazard regression analysis was repeated with the inclusion of the 'treatment' category, defined as described above. Weighted mean HbA1C <6% was still associated with increased risk of LVH development (HR 2.2, 95% CI 1.7-2.7). However, weighted mean HbA1C <=8 to 9% no longer conveyed increased risk of LVH development when medications at entry into study were accounted for (HR 1.1, 95% CI 0.9-1.3).

Weighted mean HbA1C >= 9% was still associated with significantly increased risk of HF development (weighted mean HbA1C >=9 to 10% HR 1.4, 95% CI 1.1-1.8, weighted mean HbA1C >=10% HR 1.5, 95% CI 1.1-2.1). These results are summarized in table 4.1.5. A significantly greater proportion of the LVH cases were taking insulin and/or sulphonylureas than non-LVH cases across all levels of weighted mean HbA1C (table 4.1.6).

Table 4.1.5: Risk of LVH development associated with varying levels of glycaemic control, taking baseline anti-hyperglycaemic medications into account

Variable	Hazard ratio	95% CI	P-value
Age, years	1.04	1.04-1.05	6.73x10 ⁻³²
Gender	0.85	0.75-0.96	0.01
Duration diabetes, years	1.01	1.00-1.02	0.34
SBP, mmHg	1.01	1.00-1.01	3.63x10 ⁻⁴
DBP, mmHg	0.85	0.75-0.96	5.57x10 ⁻⁴
BMI, kg/m ²	1.08	1.04-1.07	1.21x10 ⁻²¹
Creatinine category, umol/L	1.08	1.05-1.11	5.81x10 ⁻⁹
CAD	3.62	3.21-4.08	2.37x10 ⁻⁹⁷
Weighted mean HbA1C <6%	2.17	1.74-2.71	7.92x10 ⁻¹²
Weighted mean HbA1C ≥6-7%	1.00	1.00-1.00	NA (ref)
Weighted mean HbA1C ≥7-8%	0.98	0.84-1.16	0.85
Weighted mean HbA1C ≥8-9%	1.07	0.88-1.31	0.48
Weighted mean HbA1C ≥9-10%	1.38	1.07-1.79	0.01
Weighted mean HbA1C ≥10%	1.53	1.11-2.10	0.01
Insulin therapy	1.63	1.30-2.05	2.74x10 ⁻⁵
Sulphonylureas	1.56	1.30-1.87	2.22x10 ⁻⁶
Thiazolidinediones	2.90	1.07-7.83	0.04
Metformin	1.42	1.17-1.73	4.28x10 ⁻⁴
Diet control (ref)	1.00	1.00-1.00	NA (ref)

CI=confidence interval, P-value=probability value, HbA1C= glycosylated haemoglobin, CAD =coronary artery disease, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, ref=reference group

Table 4.1.6: Frequency distribution of prescribed therapy at study enrolment and LVH cases according to glycaemic control represented by weighted mean HbA1C range

Glycaemic control group	LVH status	Frequency insulin +/-or SUs (%)	Frequency metformin (%)	Frequency TZDs (%)
Weighted mean HbA1C<6%	LVH cases	31 (27.0)	15 (13.0)	2 (1.7)
	Non-LVH cases	25 (9.6)	26 (9.9)	1 (0.38)
	P-value	<0.01	0.4	NA
Weighted mean HbA1C 6-8%	LVH cases	351 (53.1)	161 (23.9)	1 (0.2)
	Non-LVH cases	982 (39.2)	782 (30.3)	10 (0.4)
	P-value	<0.01	<0.01	NA
Weighted mean HbA1C >=8%	LVH cases	318 (86.4)	30 (8.0)	1 (0.3)
	Non-LVH cases	644 (76.9)	114 (13.3)	2 (0.2)
	P-value	<0.01	0.01	NA

NA used where figures too small to allow statistically valid testing.

HbA1C=glycosylated haemoglobin, SU= sulphonylurea, TZD=thiazolidinedione, p-value=probability C and LVH development.

4.1.7 Genotype association: previously characterised SNPs associated with LVH in our cohort

Further factors influencing the presence of LVH in T2DM were also investigated. The influence of genetic factors and how these interact with glycaemic control were explored and the results are given below.

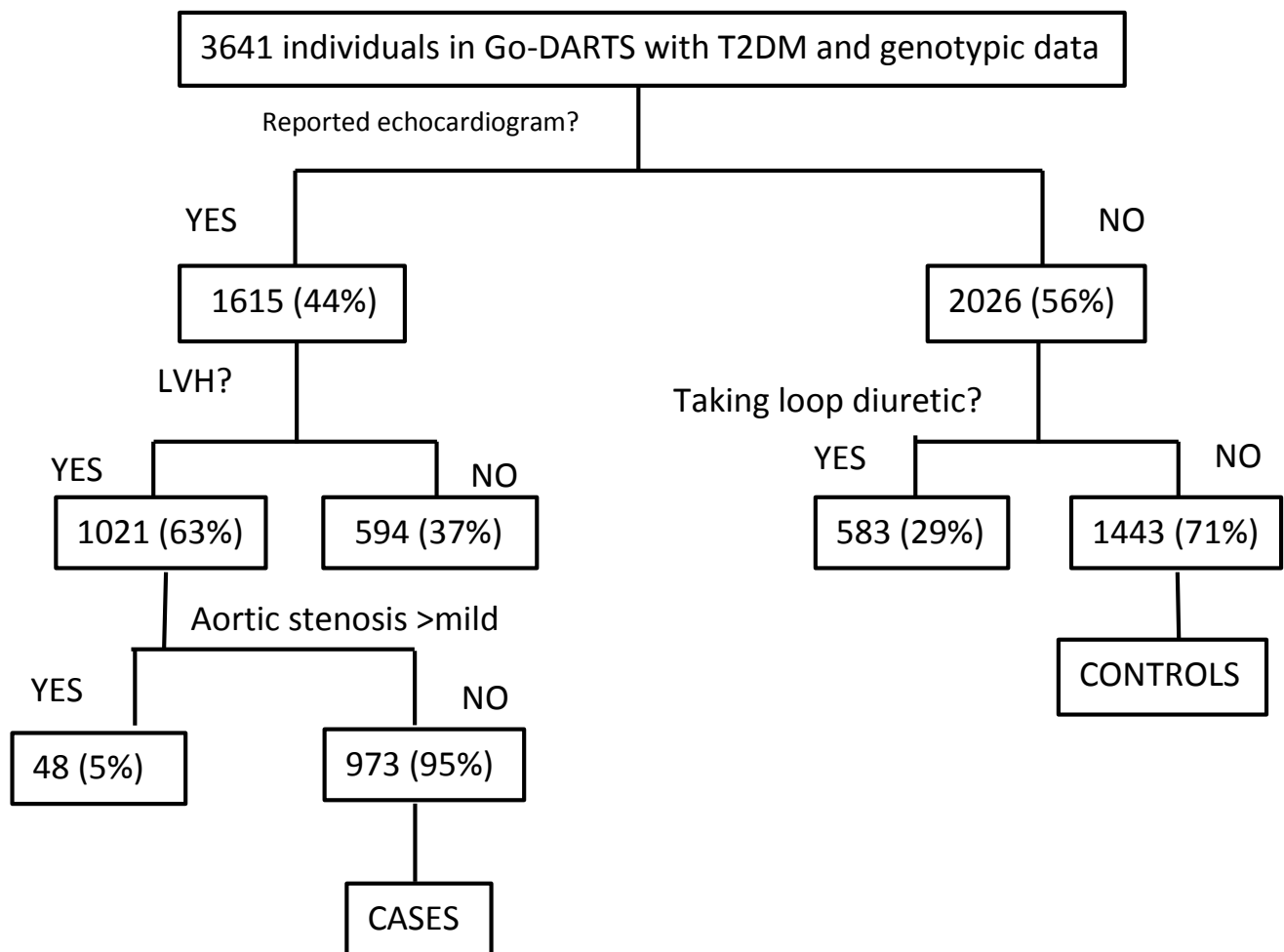
4.1.7.1 LVH-cases and non-LVH controls with available genotypic data

Fewer LVH cases and non-LVH controls were available for the genotypic association replication study, as not all individuals were genotyped. Hence, the numbers of individuals involved in this study are summarised in the flow chart below. Table 4.1.7 shows the comparison between baseline characteristics in LVH- cases and non-LVH controls with available genotypic data according to multiple logistic regression analysis. Advancing age, decreasing diastolic blood pressure, decreasing height, increasing weight and increasing HbA1C were associated with significantly increased odds ratio (OR) for LVH.

Table 4.1.7: Comparison of baseline characteristics in LVH cases versus non-LVH controls according to multiple logistic regression analysis

Variable: mean (SD)	Cases (n=973) mean (SD)	Controls (n=1443) mean (SD)	P value	OR	95% CI
Male %	52.62	58.90	0.94	1.01	0.78-1.30
Age years	66.90 (9.83)	62.15 (11.10)	<0.01	1.05	1.04-1.06
SBP mmHg	140.76 (23.07)	139.85(17.47)	0.07	1.01	1.00-1.01
DBP mmHg	75.09 (13.48)	78.19 (9.97)	<0.01	0.98	0.97-0.99
Height m	1.66 (0.09)	1.68 (0.10)	<0.01	0.04	0.01-0.15
Weight kg	85.85 (18.76)	84.00 (17.10)	<0.01	1.03	1.02-1.03
HbA1C %	7.70 (1.11)	7.63 (1.08)	<0.01	1.20	1.11-1.31

Figure 4.1.3: Flow chart summarising numbers of LVH cases and non-LVH controls taking into account the availability of genotypic data



There were 356 (15%) deaths during the median follow up period of 5.62 years (SD 2.64). Mortality in cases of LVH exceeded that of controls as shown in figure 4.1.4.1. There was a statistically significant difference in mortality comparing controls and cases ($p < 0.01$, hazard ratio 2.44, 95% CI 1.93-3.09). There was also significantly increased risk in LVH cases for composite outcome of death or hospitalization due to cardiovascular disease according to proportional hazard regression analysis ($p < 0.01$, hazard ratio 2.18, 95% CI 1.89-2.52) as shown figure 4.1.4.2.

Figure 4.1.4: Variation in survival time in cases of LVH versus non-LVH controls.

4.1.4.1 – Kaplan-Meier plot illustrating difference in years to death in LVH cases versus non-LVH controls.

4.1.4.2- Kaplan-Meier plot illustrating difference in years to death or hospitalization due to cardiovascular disease in LVH cases versus non-LVH controls.

Figure 4.1.4.1

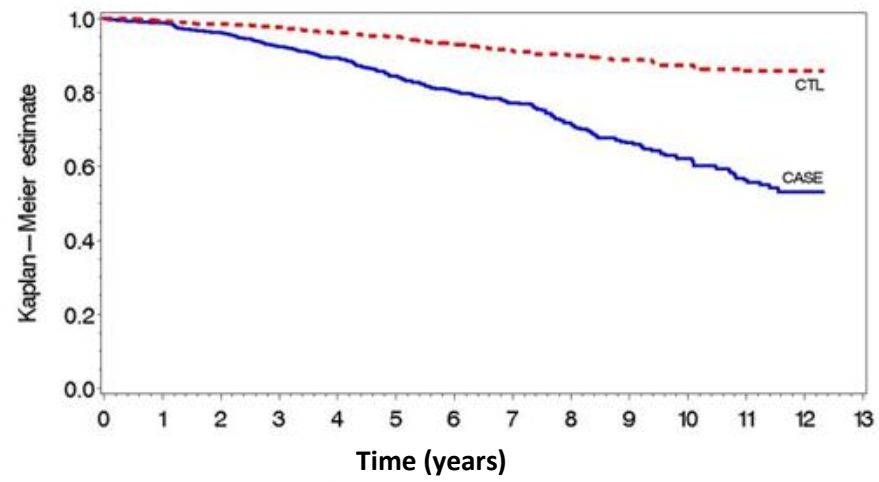
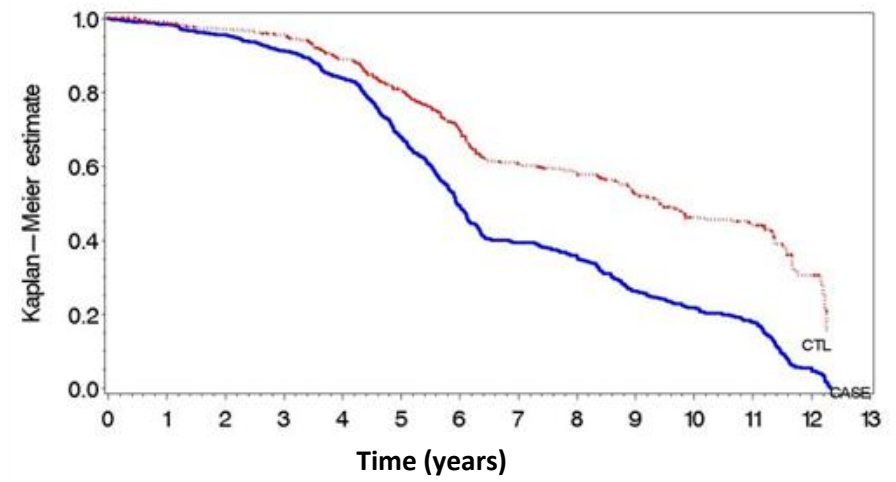


Figure 4.1.4.2



4.1.7.2 Previously characterised SNPs associated with LVH

Six out of nine SNPs previously associated with LVH through GWAS had beta values showing the same directionality in our study. Rs17132261 showed a significant association with case-control status and had a minor allele frequency (MAF) of 0.01 in our population. Our results showed every copy of the 'T' allele was associated with increased risk of LVH (table 10, $p=0.02$, beta 0.73, SE 0.31, OR 2.03, 95%CI 1.10-3.73). Rs2292462 showed a significant association with LVH and had a MAF of 0.45 in our population. Every copy of the 'G' allele was associated with decreased risk of LVH (table 10, $p=2.26 \times 10^{-3}$, beta -0.20, SE 0.06, OR 0.82, 95% CI 0.73-0.93).

Repeating the logistic regression analysis following division of cases into those with increased LV mass and those with concentric/eccentric remodelling showed rs17132261 remained significant (LV mass phenotype $p=0.01$, increased wall thickness phenotype $p=0.01$) as did rs2292462 (LV mass phenotype $p<0.01$, increased wall thickness phenotype $p<0.01$).

Rs17132261 variation did not predict mortality and morbidity, (figure 4.1.5.1, table 4.1.7) but each copy of the 'G' allele variant in rs2292462 showed a protective, dose-dependent relationship with cardiovascular hospitalization and mortality, which was attenuated but remained significant when covariates were accounted for (figure 4.1.5.2, table 4.1.7).

Results from the meta-analyses are also displayed in table 4.1.7. A total of 15 028 individuals were analysed for the SNPs published by Vasan *et al* (105) and 24 449 individuals were analysed for the SNPs published by Shah *et al* (106). Investigators within both of these studies sought to identify SNPs associated with LVH, whilst Vasan and colleagues looked at echocardiographically defined LVH, Shah and colleagues looked at electrocardiographically defined LVH. Notably, the combined p -values for rs7910620, rs17132261, rs2292462 and rs4966014 exceeded the threshold for genome wide significance ($p<5.0 \times 10^{-8}$). Although not significant in our study alone the significance for

rs4966014 had been previously published as 8×10^{-7} and achieved genome wide significance (1.35×10^{-8}) after the addition of our data. This SNP lies within an intron of the IGF1R gene.

4.1.7.3 Genotypic score calculation and association with LVH

Logistic regression analysis showed genotypic scoring based on gene score 1 did not predict LVH. However, gene scores 2 and 3 were significant predictors of LVH, as summarised in table 4.1.8.

Table 4.1.8: Comparison between previously published SNP results and results obtained in this study

SNP	Initially published by	E A	EAF	Hardy-Weinberg equilibrium	Published beta	Published P value	Study beta	Study SE	Study p values	Meta-p value	Effect on LVH P value (SE)	Effect on LVH Hazard ratio	Effect on LVH 95% CIs for HR
rs17568359	Vasan et al	C	0.06	0.35	-4.78	8.53x10 ⁻⁸	-0.16	0.13	0.23	1.75x10 ⁻⁶	0.29 (0.10)	0.90	0.73-1.10
rs7565161	Vasan et al	A	0.44	0.75	-3.01	3.19x10 ⁻⁷	0.09	0.07	0.19	5.35x10 ⁻⁶	0.69 (0.06)	0.98	0.87-1.09
rs7910620	Vasan et al	G	0.01	1.00	0.17	5.62x10 ⁻⁹	-0.44	0.42	0.29	1.45x10 ⁻⁸	0.74 (0.58)	0.83	0.27-2.61
rs2059238	Vasan et al	A	0.23	0.31	-0.02	1.89x10 ⁻⁷	-0.06	0.07	0.45	5.07x10 ⁻⁷	0.56 (0.05)	1.03	0.93-1.15
rs17132261	Vasan et al	T	0.01	1.00	0.06	3.36x10 ⁻⁷	0.73	0.31	0.02	1.03x10 ⁻⁸	0.54 (0.20)	1.13	0.76-1.68
rs6797133	Shah et al	A	0.39	0.66	-3.7	1.2x10 ⁻⁷	3.73x10 ⁻³	0.06	0.95	2.42x10 ⁻⁷	0.74 (0.05)	1.02	0.92-1.12
rs2292462	Shah et al	G	0.45	0.06	-218.6	3.2x10 ⁻⁹	-0.20	0.06	2.26x10 ⁻³	5.86x10 ⁻¹⁰	<0.01 (0.05)	0.87	0.80-0.96
rs4966014	Shah et al	C	0.31	0.33	-181.8	1.3x10 ⁻⁷	-0.02	0.07	0.74	3.35x10 ⁻⁸	0.13 (0.06)	0.92	0.82-1.03
rs2290893	Shah et al	G	0.64	0.77	-201.4	3.7x10 ⁻⁸	-1.57x10 ⁻³	0.06	0.98	1.70x10 ⁻⁷	0.17 (0.05)	0.94	0.85-1.03

Figure 4.1.5.1 – Kaplan-Meier showing difference in years to death or hospitalization due to cardiovascular disease in carriers of the ‘T’ variant of rs17132261 (T_1) versus non-carriers (T_0). 4.1.5.2– Kaplan –Meier showing how difference in years to death or hospitalization due to cardiovascular disease varies between carriers of the ‘G’ variant of rs2292462 (G_1), homozygotes for the ‘G’ variant (G_2) and non-carriers (G_0).

Figure 4.1.5.1

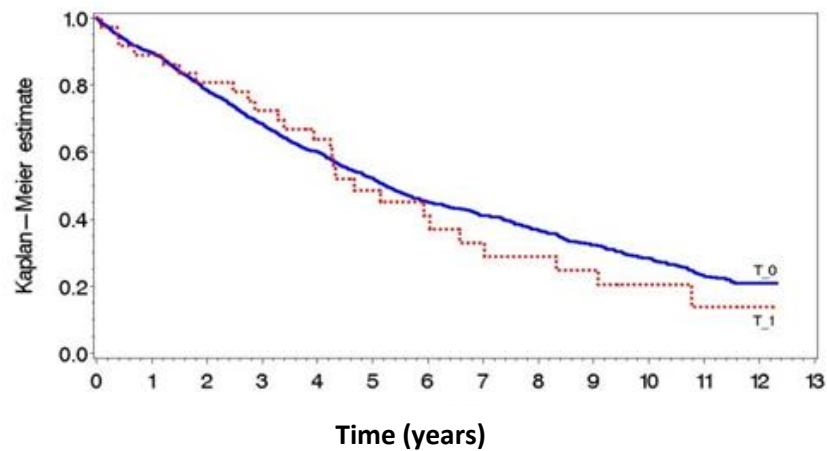


Figure 4.1.5.2

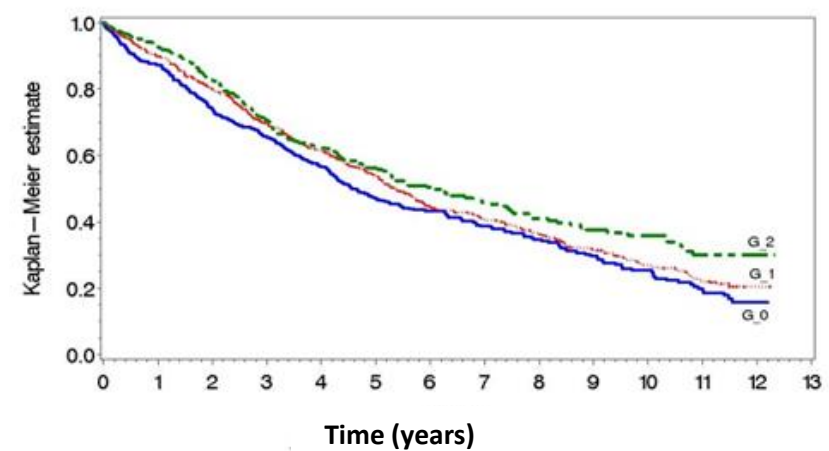


Table 4.1.9: Association between gene scoring based on previously published SNPs and LVH

Gene score	OR	95% Confidence intervals	P value
Vasan SNPs	1.01	0.94-1.07	0.87
Shah SNPs	1.10	1.03-1.17	4.52×10^{-3}
Vasan-Shah SNPs	1.09	1.03-1.16	6.24×10^{-3}

4.2: HF in T2DM

The data below show the results from the analyses looking at factors influencing development of HF in T2DM. This allows comparison between the relationships between glycaemic control and the development of HF in T2DM and glycaemic control and LVH.

4.2.1 Frequencies of HF-cases and non-HF cases

A total of 701 (8.07%) out of the 8 683 individuals developed HF during the study period (mean 5.53 years, standard deviation 2.81 years, figure 4.2.1). These comprised 196 cases of HF based on echocardiography data and 505 based on previous hospital admission with HF. Of the 505 cases classed as having HF based on hospital admission, 216 subsequently had an echocardiogram showing LV systolic impairment. Thus, a total of 412 (59%) cases had HF with reduced ejection fraction. Of the remaining 289 patients, 217 (31%) had preserved ejection fraction on echocardiography, the echocardiographers were unable to comment on LV function in 16 cases (2%) and 56 HF cases (8%) did not undergo echocardiography. Out of the 701 individuals with HF and T2DM, 683 HF cases could be matched to non-HF controls for age within 2 years, date of diabetes diagnosis within 2 years and gender. These figures are summarized in the flow chart below:

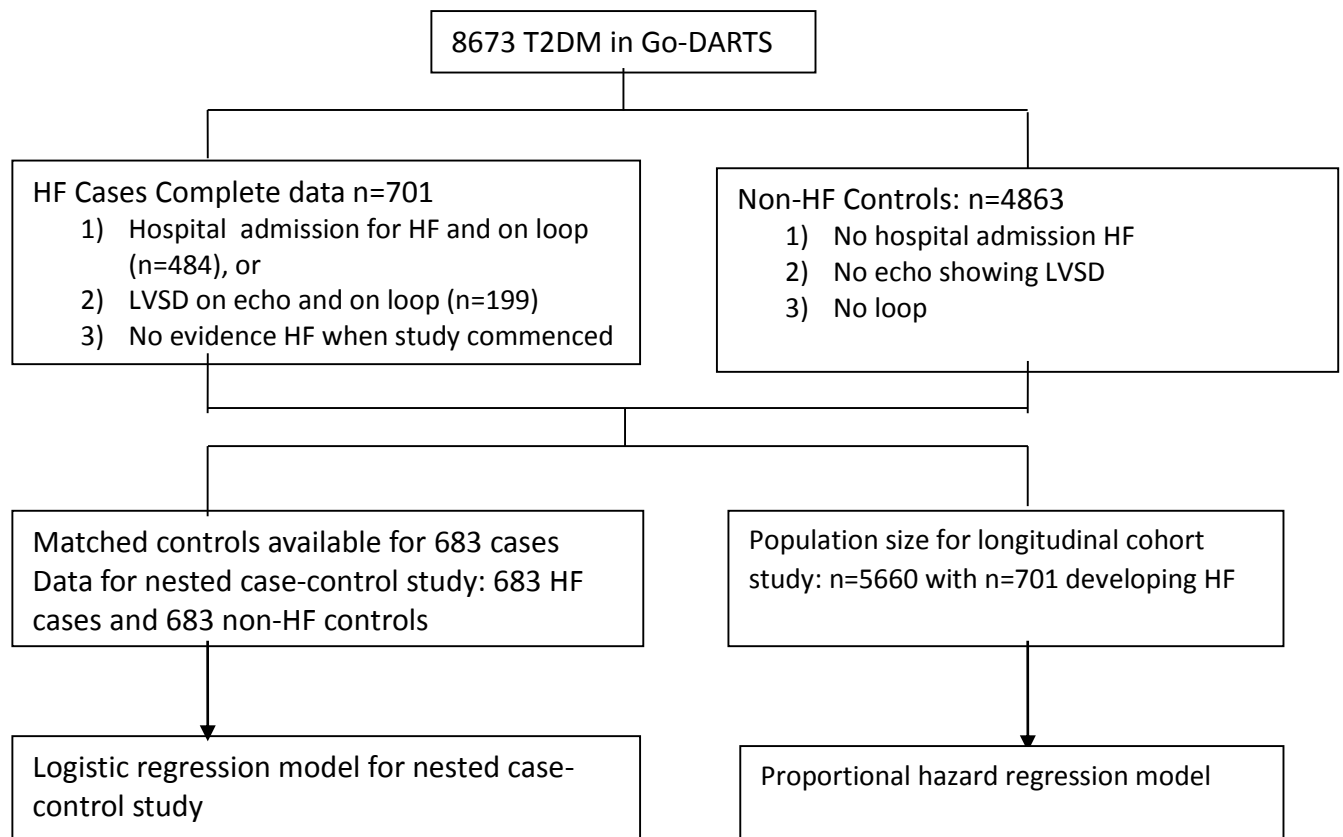


Figure 4.2.1 shows the identification of HF-cases and non-HF cases for the nested case-control study and prospective cohort study

4.2.2 Clinical characteristics: HF cases and non-HF controls nested case-control study

Clinical characteristics for all individuals who developed HF and those who did not develop HF are summarised in the table below:

Table 4.2.1: Clinical characteristics of HF cases and non-HF controls, nested case-control study

Variable	HF cases	Controls	Significance, p=
N	683	683	NA
Number HbA1C measures (SD)	27.33 (15.11)	24.33 (12.32)	<0.01
Time period HbA1C measured, yrs (SD)	3.77 (2.80)	3.01 (3.93)	<0.01
Age T2DM diagnosis, yrs (SD)	62.40 (10.83)	62.47 (10.76)	NA
% Males	391 (57.25)	391 (57.25)	NA
Duration T2DM, yrs (SD)	11.88(6.97)	11.74 (6.88)	NA
% CAD	463 (67.79)	159 (23.35)	<0.01
SBP, mmHg (SD)	142.97 (21.36)	140.93 (17.47)	0.05
BMI, kg/m ² (SD)	31.53 (5.94)	28.64 (4.91)	<0.01
creatinine, umol/L (SD)	108.23 (48.19)	95.40 (23.94)	<0.01
Weighted mean HbA1C % (SD)	7.67 (1.64)	7.05 (2.85)	<0.01
% Insulin at HF diagnosis	264 (38.65)	106 (15.57)	<0.01
% SUs at HF diagnosis	203 (29.72)	231 (33.92)	0.10
% Metformin at HF diagnosis	194 (28.40)	251 (36.86)	<0.01
% TZD at HF diagnosis	44 (6.44)	53 (7.78)	0.34
% Diet control at HF diagnosis	124 (18.16)	233 (34.21)	<0.01

The matching process was deemed successful as there were equal numbers of males and females in each group and there was no significant difference in age at diabetes diagnosis or duration of diabetes between the 2 groups. There was a significant association between HF development and the following variables: increasing number of HbA1C measures, SBP, BMI, creatinine and weighted mean HbA1C. There was also a significant association between HF development and a history of

CAD and baseline insulin therapy. These covariates were taken forward for use in the logistic regression model.

There were significant differences between HF-cases and those who did not develop HF for all variables examined except prescription of TZDs at study enrolment. All significant variables were taken forward for use as covariates in subsequent regression modelling of HF.

4.2.3 Independent risk factors for HF development in T2DM: nested case-control study

Conditional logistic regression modelling showed CAD, BMI, creatinine and weighted mean HbA1C were significant independent predictors of HF development. In this model, weighted mean HbA1C was categorised into 6 groups: <6%, ≥6 to 7%, ≥7 to 8%, ≥8 to 9%, ≥9 to 10% and greater than or equal to 10%. Weighted mean HbA1C ≥6-7% was taken as the reference range. Weighted mean HbA1C ≥8% was associated with increased risk of HF development compared to the reference range (table 4.2.2). The frequency of cases and controls within the different weighted mean HbA1C categories is shown in table 4.2.6.

Simple logistic regression modelling in the group of patients with CAD and in the group of patients without CAD showed that BMI, creatinine and weighted mean HbA1C were associated with HF in T2DM irrespective of CAD status (table 4.2.4). Weighted mean HbA1C ≥7 to 8%, ≥8 to 9% and ≥10% were associated with increased risk of HF development in those with CAD. Weighted mean HbA1C ≥8% was associated with increased risk of HF development in those without CAD.

Table 4.2.2: Independent predictors of HF in T2DM according to logistic regression modelling

Variable	OR	95% CI	P value
CAD	6.81	4.92-9.42	4.43×10^{-31}
SBP	1.00	1.00-1.01	0.34
BMI, kg/m ²	1.11	1.07-1.14	5.88×10^{-11}
Creatinine, umol/L	1.16	1.09-1.23	6.99×10^{-5}
M_HbA1C <6 %	0.89	0.53-1.49	0.65
M_HbA1C >=6-7%	1.00	NA (ref)	NA (ref)
M_HbA1C >=7-8%	1.33	0.92-1.93	0.13
M_HbA1C >=8-9%	1.80	1.15-2.83	0.01
M_HbA1C >=9-10%	2.54	1.30-4.99	0.01
M_HbA1C >=10%	2.90	1.23-6.85	0.01

4.2.4 Proportional hazard regression analysis: optimum range for glycaemic control with respect to HF

Proportional hazard regression analysis showed the hazard ratio of developing HF was significantly increased with weighted mean HbA1C below 6% and above or equal to 8% as shown in Figure 4.2.2 and Table 4.2.3. Hazard ratio increased significantly with HbA1C greater than or equal to 8%.

However, weighted mean HbA1C below 6% was associated with greater risk of HF development than weighted mean HbA1C >=8 to 9% (HbA1C below 6% HR 2.2, 95% CI 1.6-2.9, $p=1.4 \times 10^{-7}$, HbA1C >=8 to 9% HR 1.6, 95% CI 1.2-2.0, $p=3.2 \times 10^{-4}$).

Age, duration of diabetes, history of CAD, BMI, creatinine category and SBP were all independent predictors of HF development in T2DM according to the proportional hazard regression model (Table 4.2.3).

Figure 4.2.2: Relationship between weighted mean glycosylated haemoglobin (HbA1C) and the development of heart failure in type 2 diabetes, including age, duration of diabetes, gender, history of coronary artery disease, body mass index, creatinine, systolic and diastolic blood pressure as covariates

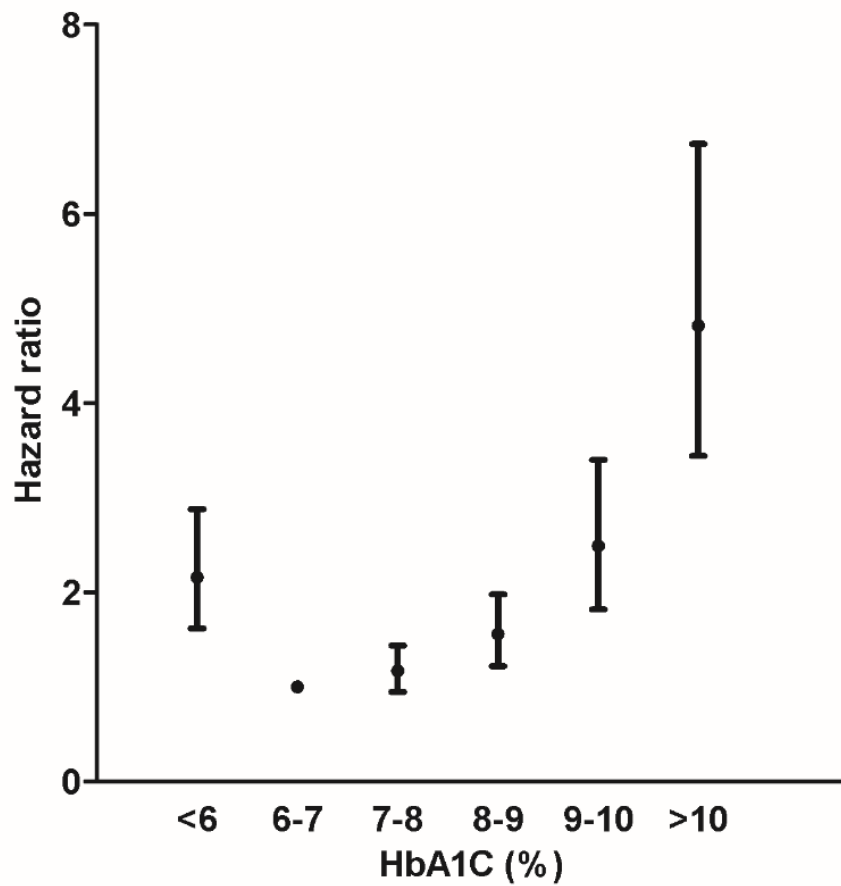


Table 4.2.3: Risk of heart failure development associated with varying levels of glycaemic control

Variable	Hazard ratio	95% CI	P value
Age at enrolment, yrs	1.1	1.1-1.1	7.2×10^{-53}
Duration T2DM, yrs	1.0	1.0-1.0	0.06
Number of HbA1C measures	1.0	1.0-1.0	0.1
Gender	1.0	0.9-1.2	0.7
CAD	5.1	4.3-6.0	1.3×10^{-84}
BMI, kg/m ²	1.1	1.1-1.1	1.5×10^{-33}
Creatinine category, umol/L	1.1	1.1-1.2	9.7×10^{-17}
SBP, mmHg	1.0	1.0-1.0	0.02
DBP, mmHg	1.0	1.0-1.0	0.02
Weighted mean HbA1C <6%	2.2	1.7-2.9	5.4×10^{-8}
Weighted mean HbA1C ≥6-7%	1.0	1.00-1.0	NA (ref)
Weighted mean HbA1C ≥7-8%	1.2	1.0-1.5	0.1
Weighted mean HbA1C ≥8-9%	1.6	1.2-2.0	2.9×10^{-4}
Weighted mean HbA1C ≥9-10%	2.5	1.8-3.4	1.2×10^{-8}
Weighted mean HbA1C ≥10%	5.0	3.6-7.0	5.9×10^{-21}

CI=confidence interval, P-value=probability value, HbA1C= glycosylated haemoglobin, yrs=years, T2DM=type 2 diabetes mellitus, CAD =coronary artery disease, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, SU=sulphonylurea, TZD=thiazolidinedione, ref=reference group

4.2.5 Role of CAD in the relationship between glycaemic control and incident HF

Proportional hazard regression analysis was repeated excluding CAD from the list of covariates to investigate whether the relationship between HF and glycaemic control was driven by an association between CAD and glycaemic control. There was a significant increase in the risk of developing HF with weighted mean HbA1C <6% in both models (HR 2.2 with the inclusion of CAD, HR 2.5 when CAD was not taken into account). There was a significant increase in the risk of HF development with all

weighted mean HbA1C categories greater than or equal to 7% (HbA1C ≥ 7 to 8% HR 1.2, 95% CI 1.0-1.5, HbA1C ≥ 8 to 9%

HR 1.8, 95% CI 1.4-2.3, HbA1C ≥ 9 to 10% HR 3.0, 95% CI 2.2-4.1, HbA1C $\geq 10\%$ HR 6.6, 95% CI 4.7-9.2) whereas weighted mean HbA1C ≥ 7 to 8% was not associated with significantly increased risk of HF development when CAD was included in the model (HR 1.2, 95% CI 0.9-1.4). These results are summarised in table 4.5b. Overall, exclusion of CAD from the list of covariates did not make a difference to the pattern seen in the relationship between weighted mean HbA1C and HF development. This mirrors the findings in the above analysis where LVH was modelled with the inclusion and exclusion of CAD.

Variable	Hazard ratio	95% CI	P-value
Age at enrolment, yrs	1.1	1.1-1.1	4.9×10^{-70}
Duration T2DM, yrs	1.0	1.0-1.0	5.4×10^{-3}
Number of HbA1C measures	1.0	1.0-1.0	4.5×10^{-4}
Gender	0.9	0.8-1.1	0.5
CAD	NA	NA	NA
BMI, kg/m ²	1.1	1.1-1.1	1.3×10^{-39}
Creatinine category, umol/L	1.1	1.1-1.2	4.1×10^{-18}
SBP, mmHg	1.0	1.0-1.0	0.01
DBP, mmHg	1.0	1.0-1.0	2.9×10^{-5}
Weighted mean HbA1C <6%	2.5	1.9-3.3	3.0×10^{-10}
Weighted mean HbA1C $\geq 6-7\%$	1.0	1.0-1.0	NA (ref)
Weighted mean HbA1C $\geq 7-8\%$	1.2	1.0-1.5	0.04
Weighted mean HbA1C $\geq 8-9\%$	1.8	1.4-2.3	1.8×10^{-6}
Weighted mean HbA1C $\geq 9-10\%$	2.9	2.1-4.0	1.2×10^{-11}
Weighted mean HbA1C $\geq 10\%$	6.8	4.9-9.6	3.8×10^{-29}

Table 4.2.4: Risk of HF development associated with varying levels of glycaemic control, excluding CAD from the covariates

CI=confidence interval, P-value=probability value, HbA1C= glycosylated haemoglobin, yrs=years, T2DM=type 2 diabetes mellitus, CAD =coronary artery disease

4.2.6 Role of anti-hyperglycaemic agents in the relationship between glycaemic control and incident HF

Prescription of anti-hyperglycaemic medications also differed significantly in the HF cases relative to non-HF patients. There were significant differences in prescribing rates for insulin, sulphonylureas, metformin and in those categorised as diet-control. Prescriptions of insulin and sulphonylureas were higher in patients who developed HF whilst metformin prescriptions and diet-control were higher in patients without HF compared to HF cases.

The proportional hazard regression analysis was repeated with the inclusion of the 'treatment' category, defined as described above. Weighted mean HbA1C <6% was still associated with HF development (HR 2.5, 95% CI 1.8-3.3). However, weighted mean HbA1C ≥8-9% no longer conveyed increased risk of HF development when medications at entry into study were accounted for (HR 1.2, 95% CI 0.9-1.5).

Weighted mean HbA1C ≥9% was still associated with significantly increased risk of HF development (weighted mean HbA1C ≥9 to 10% HR 1.7, 95% CI 1.2-2.4, weighted mean HbA1C ≥10% HR 3.5, 95% CI 2.4-5.0). These results are summarized in table 4.5c. A significantly greater proportion of the HF cases were taking insulin and/or sulphonylureas than non-HF cases across all levels of weighted mean HbA1C (table 4.2.5).

Table 4.2.5: Risk of HF development associated with varying levels of glycaemic control, taking baseline anti-hyperglycaemic medications into account

Variable	Hazard ratio	95% CI	P value
Age at enrolment, yrs	1.1	1.1-1.1	5.5×10^{-55}
Duration T2DM, yrs	1.0	1.0-1.0	0.7
Number of HbA1C measures	1.0	1.0-1.0	0.5
Gender	1.0	0.9-1.2	0.8
CAD	5.1	4.3-6.0	6.7×10^{-84}
BMI, kg/m ²	1.1	1.1-1.1	1.1×10^{-29}
Creatinine category, umol/L	1.1	1.1-1.2	2.7×10^{-16}
SBP, mmHg	1.0	1.0-1.0	0.03
DBP, mmHg	1.0	1.0-1.0	0.04
Weighted mean HbA1C <6%	2.5	1.8-3.3	6.7×10^{-10}
Weighted mean HbA1C ≥6-7%	1.0	1.0-1.0	NA (ref)
Weighted mean HbA1C ≥7-8%	1.0	0.8-1.2	0.9
Weighted mean HbA1C ≥8-9%	1.2	0.9-1.5	0.2
Weighted mean HbA1C ≥9-10%	1.7	1.2-2.4	1.5×10^{-3}
Weighted mean HbA1C ≥10%	3.5	2.4-5.0	1.3×10^{-11}
Insulin therapy	2.4	1.8-3.3	9.5×10^{-9}
Sulphonylureas	1.8	1.4-2.4	3.7×10^{-6}
Metformin	1.6	1.2-2.2	7.1×10^{-4}
Thiazolidinediones	2.3	1.3-3.9	2.9×10^{-3}
Diet control (ref)	1.0	1.0-1.0	NA (ref)

CI=confidence interval, P-value=probability value, HbA1C= glycosylated haemoglobin, yrs=years, T2DM=type 2 diabetes mellitus, CAD =coronary artery disease, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, SU=sulphonylurea, TZD=thiazolidinedione, ref=reference group

Table 4.2.6: Frequency distribution of prescribed therapy at study enrolment and HF cases according to glycaemic control represented by weighted mean HbA1C range

Glycaemic control group	HF status	Frequency insulin +/or SUs (%)	Frequency metformin (%)	Frequency TZDs (%)
Weighted mean	HF cases	18/68 (26.5)	6/68 (8.8)	1/68 (1.5)
HbA1C<6%	Non-HF cases	27/377 (7.2)	33/377 (8.8)	3/377 (0.8)
	P value	<0.01	0.2	NA
Weighted mean	HF cases	222/387 (57.4)	81/387 (20.9)	8/387 (2.1)
HbA1C >=6-8%	Non-HF cases	1258/3417 (36.8)	999/3417 (29.2)	100/3417 (2.9)
	P value	<0.01	<0.01	0.3
Weighted mean	HF cases	215/246 (87.4)	15/246 (6.1)	7/246 (2.9)
HbA1C >=8%	Non-HF cases	808/1069 (75.6)	141/1069 (13.2)	27/1069 (2.5)
	P value	<0.01	<0.01	0.7

HbA1C=glycosylated haemoglobin, HF=heart failure, TZD=thiazolidinedione

4.2.7 HbA1c variability and incident HF

Intra-individual mean (HbA1c-MEAN) and its adjusted standard deviation (HbA1c-SD) were included in the proportional hazard model as above. HbA1c variability (HbA1c-SD) was independently and significantly associated with incident HF (HR=0.801 CI 0.74-0.85, P-value<0.0001), with less variability in HbA1c having a protective effect on incident HF.

4.2.8 HF development and glycaemic control SNPs

Rs560887, rs7944584 and rs10885122SNPs, previously associated with fasting glucose, were associated with HF development in our cohort at the 1 in 20 significance level, as tabulated below

(table 4.2.7). No SNPs previously associated with HbA1c were associated with HF development in our cohort (table 4.2.8).

Table 4.2.7 Association between HF development and SNPs linked to glycaemic control by Dupuis *et al* (140)

SNP	EA	EAf	HR	95% CI	P-value	HR With drugs	CI with drugs	P value with drugs
Rs56088	T	0.29	1.19	1.00-1.41	0.05	1.20	1.01-1.43	0.03
Rs10830963	G	0.28	0.92	0.78-1.10	0.36	0.92	0.77-1.09	0.32
Rs4607517	A	0.18	1.12	0.91-1.37	0.29	1.12	0.91-1.37	0.30
Rs2191349	G	0.44	1.08	0.92-1.27	0.35	1.08	0.92-1.28	0.34
Rs780094	T	0.37	1.09	0.93-1.28	0.27	1.10	0.93-1.29	0.27
Rs11708067	G	0.22	1.20	1.00-1.45	0.05	1.20	0.99-1.44	0.06
Rs7944584	T	0.26	0.83	0.69-1.00	0.05	0.82	0.69-0.99	0.04
Rs10885122	T	0.0003	1.31	1.02-1.68	0.04	1.33	1.03-1.71	0.03
Rs174550	C	0.35	0.96	0.80-1.13	0.59	0.97	0.82-1.15	0.73
Rs11605924	A	0.48	1.10	0.94-1.29	0.23	1.11	0.95-1.30	0.20
Rs11920090	A	0.12	0.94	0.73-1.20	0.60	0.95	0.74-1.22	0.70
Rs7034200	A	0.47	0.94	0.80-1.10	0.45	0.93	0.79-1.09	0.36
Rs340874	T	0.40	0.90	0.76-1.07	0.23	0.92	0.77-1.09	0.33
Rs11071657	G	0.37	0.95	0.79-1.14	0.58	0.95	0.79-1.14	0.60
Rs11558471	G	0.30	1.01	0.85-1.22	0.88	0.99	0.82-1.19	0.89
Rs13266634	T	0.29	1.02	0.85-1.22	0.85	0.99	0.83-1.19	0.93
Rs4506565	T	0.38	1.00	0.85-1.19	0.98	1.00	0.84-1.18	0.96

Table 4.2.8 Association between development of HF and SNPs associated with glycaemic control by Soranzo *et al* (142)

SNP	EA	EAF	HR	95% CI	P-value	WD HR	WD CI	WD P value
Rs2779116	No data available for this SNP							
Rs552976	A	0.34	1.13	0.96-1.34	0.14	1.16	0.98-1.37	0.08
Rs1800562	A	0.09	0.91	0.69-1.20	0.50	0.92	0.69-1.22	0.56
Rs1799884	T	0.18	1.10	0.90-1.36	0.35	1.10	0.89-1.35	0.37
Rs6474359	C	0.04	1.34	0.94-1.91	0.10	1.32	0.93-1.89	0.12
Rs4737009	A	0.24	0.95	0.78-1.15	0.57	0.96	0.79-1.16	0.65
Rs16926246	T	0.11	0.98	0.75-1.30	0.91	0.93	0.70-1.23	0.61
Rs1387153	T	0.30	0.87	0.73-1.04	0.12	0.87	0.73-1.04	0.13
Rs7998202	G	0.12	1.00	0.74-1.37	0.98	0.13	0.75-1.39	0.87
Rs1046896	T	0.31	0.86	0.73-1.02	0.09	0.85	0.72-1.01	0.07
Rs855791	A	0.42	0.92	0.77-1.09	0.33	0.93	0.78-1.10	0.39

Chapter 5: Discussion

5.1 Rate of HF development in the Go-DARTS cohort

Eight per cent of patients with T2DM in our cohort developed HF over 5.53 years, which compares well with the 13.2% over 9.9 years quoted by Lind and colleagues (62). HF incidence in our cohort exceeds that of the general population of Scotland, which was approximately 0.15% per year based on hospital admission data (175). This may be partially explained by the increased incidence of HF in T2DM (9,18,19). It is also possible that combining admission data and echocardiographic data leads to increased sensitivity in capturing HF development, although this is speculative at this stage.

5.2 Prevalence of LVH in the Go-DARTS cohort

Sixty-one per cent of patients with T2DM undergoing echocardiography in our cohort had LVH, which compares reasonably to previous studies quoting 71% (176). Data regarding prevalence of LVH in the general population are unavailable as, despite its association with poor prognosis (177), LVH is a largely asymptomatic phenomenon and is usually only discovered during investigations for other pathologies/ disease processes.

5.3 Glycaemic control and the development of HF and LVH in T2DM

Firstly, we have shown that weighted mean HbA1C $\geq 8\%$ was associated with HF development in a progressive non-linear relationship. Secondly, we found that weighted mean HbA1C $< 6\%$ was also associated with an increased HF risk (HR 2.2). These findings suggest that both overly strict glycaemic control and chronic hyperglycaemia predicted HF development in our cohort, forming a J shaped relationship.

Currie *et al* (178) had previously reported a U-shaped relationship between mean HbA1C and all-cause mortality and cardiac events in a retrospective study of patients with T2DM utilizing the UK General Practice Research Database (GPRD). Their analysis showed HbA1C around 7.5% was associated with the lowest all-cause mortality and lowest progression to large vessel disease events (myocardial infarction, stroke, coronary revascularisation or angina), although they did not look at the development of HF. Our study extends these findings, showing the U/J shaped relationship between mean HbA1C and cardiovascular risk extends to HF development.

Lind and colleagues used the Swedish National Diabetes Registry to explore the relationship between mean HbA1C and the risk of HF. They reported that individuals with mean HbA1C > 7% were at increased risk of developing HF. Their results showed 'no further reduction in risk' in patients with HbA1C < 6% (42mmol/mol) compared with those with HbA1C 6-7%. However, they did demonstrate an increased risk of HF hospitalisation in patients with mean HbA1C < 6% compared to mean HbA1C 6-7% in their model that accounted for the greatest number of confounding factors (62). These findings support a J shaped relationship between weighted mean HbA1C and risk of incident HF, in-keeping with the UK GPRD study (178) described above. This also fits with data on mortality and vascular events from recent trials investigating intensive versus standard glycaemic control and vascular outcomes (63–65).

5.3.1 Overly strict glycaemic control and the development of both LVH and HF

The J shaped relationship between weighted mean HbA1C and the risk of developing both LVH and HF merits discussion. Multiple factors may promote LVH and HF development in patients with weighted mean HbA1C < 6%. Adverse 'off-target' drug effects from anti-hyperglycaemic medications may account for some of the incident HF cases in the group of individuals with weighted mean HbA1C < 6%. Insulin, sulphonylureas and TZDs have been associated with the development of HF and both insulin and TZDs promote fluid retention (66,69). In our study, insulin appeared to pose the

greatest risk (HR 2.4), according to the calculated hazard ratios (Table 4.2c), followed by TZDs (HR 2.3), sulphonylureas (HR 1.8) and metformin (HR 1.6).

Insulin use was also associated with LVH development in the group of individuals with HbA1C <6%. This is consistent with findings by Jankovic *et al*, who looked at myocardial size and lipid content in 18 individuals with T2DM who were commenced on insulin therapy. They found insulin therapy was associated with increased myocardial lipid content and increased myocardial mass (179).

Other studies have shown sulphonylureas and TZDs were associated with relatively increased LV mass. Lee *et al* showed glibenclamide was associated with increased LV mass in individuals with T2DM. This was attenuated by either switching to gliclazide or adding nicorandil to glibenclamide. The authors argue this indicates the increase in LV mass associated with glibenclamide is mediated through cardiac potassium-ATP (K-ATP) channel blockade since gliclazide does not block K-ATP channels and nicorandil activates K-ATP channels. Gliclazide may also have a cardio-protective role over glibenclamide as it reduces oxidative stress (180).

Roes and colleagues investigated the role of rosiglitazone in LVH development. Subjects with the metabolic syndrome were randomized either to lifestyle interventions and rosiglitazone treatment or lifestyle intervention and placebo. Lifestyle intervention reduced LV mass as measured by cardiac MRI (CMR). This reduction in LV mass was attenuated by rosiglitazone therapy (178). This contrasts with previous work showing rosiglitazone was not associated with adverse cardiac remodelling assessed by echocardiography (181). This may reflect the greater accuracy of CMR in cardiac structural assessment.

Within the group of individuals with weighted mean HbA1C<6%, a significantly higher proportion who developed LVH and HF were taking insulin or sulphonylureas at baseline. There is some evidence in the literature to suggest metformin is protective against adverse cardiac remodelling in the form of LVH. In a mouse model of LVH created by trans-aortic constriction, sustained treatment with metformin was associated with reduced LV posterior wall thickness compared to placebo.

Interestingly, metformin was not associated with reduced posterior wall thickness in mice with a mutation in the cardiac K-ATP channel, suggesting metformin exerts its cardio-protective action at least partly through K-ATP channel activation (182).

The above evidence suggests increased prescription of insulin, sulphonylureas and TZDs may partly explain the increased incidence of HF in type 2 diabetics with weighted mean HbA1C<6%. It should be recognised that there are confounding factors: insulin therapy is usually a 'last resort' in T2DM and therefore a marker of poor control. It is possible that previous poor glycaemic control is actually responsible for the associated increased risk of LVH and HF development in those taking insulin.

However, it is not possible to draw firm conclusions regarding the role of anti-hyperglycaemic agents from this observational study. A randomized control trial would be required to resolve this issue, which may not be ethically feasible given this data and the data from preceding RCTs (63–65).

The other potential mechanism may be related to hypoglycaemia, a well-established side effect of both insulin and sulphonylureas (183). Hypoglycaemia is an independent cardiovascular risk factor (184,185). Hypoglycaemia may induce oxidative stress, stimulate the neuroendocrine system including the sympathetic nervous system, cause vascular dysfunction including endothelial dysfunction and increased arterial stiffness (186) and reduce myocardial oxygen utilization efficiency (187), presenting several potential mechanisms through which symptomatic and asymptomatic episodes of hypoglycaemia may increase the risk of HF development. Pro-inflammatory and pro-thrombotic effects and aggravation of pre-clinical atherosclerosis may also contribute (76,78,188). The dangers of moderate hypoglycaemia are an on-going area of research.

Although HbA1C is widely accepted as the most accurate measure of medium-term glycaemic control in T2DM (189), and mean HbA1C is well-recognised as an accurate measure of long-term control (159), neither of these measures provide any information on the frequency of hypoglycaemic episodes an individual has had. It is possible individuals with lower weighted mean HbA1C actually

have swinging blood glucose levels and are more prone to hypoglycaemic events as a result of driving a low HbA1C with multiple therapeutic agents.

Glycaemic variation may play a key role in HF development in individuals with overly strict control. Monnier and colleagues showed that post-prandial hyperglycaemia was the predominant cause of HbA1C exceeding normal levels in individuals with HbA1C below the 8-9% range and pre-prandial hyperglycaemia occurs largely in individuals with HbA1C>9% (190). This implies individuals with lower HbA1C levels are likely to have glycaemic fluctuations comparable to individuals with higher HbA1C levels, as suggested by Kilpatrick (191).

Research looking at glycaemic variation has provided evidence supporting a link between increased glycaemic variation and increased cardiac risk. Di Flaviani *et al* performed continuous glucose monitoring on 26 individuals with T2DM for 24 hours, then performed an echocardiogram the following day. Analysis showed glycaemic variation was positively associated with LV mass. Increased LV mass was also associated with increase in markers of oxidative stress. The authors suggest increased oxidative stress may be the means by which increased glycaemic variation promotes LVH (192). No studies to date have looked at how glycaemic variation influences the risk of HF development in T2DM. However, the hypothesis that glycaemic variation increased the risk of HF and LVH in our cohort is purely conjecture. Further work is needed in this area; studies using continuous blood glucose monitoring or patient diaries may provide further insight.

However, since time-dependent analysis was not carried out, discussion regarding the 'off-target' effects of diabetic medications or adverse effects of hypoglycaemia must remain speculative and firm conclusions cannot be made from this study.

Alternatively, greater HF incidence in patients with lower weighted mean HbA1C may suggest this is a group of people with poor dietary intake. Although our analysis does not provide information regarding the proportion of patients categorised as 'diet-control' who ultimately took drug-therapy,

it suggests that over-use of anti-hyperglycaemic agents is unlikely to be the only mechanism driving HF development in this group.

Our findings should be considered in the wider context of the 'obesity paradox' described within cardiovascular disease (193). This phenomenon was first described by Gruberg *et al* in the context of patients with CAD undergoing percutaneous coronary intervention. Normal weight individuals were at increased risk of both in-hospital complications and one year post procedure mortality and relative to over-weight individuals (BMI 25-30) and obese individuals (BMI greater than 30) despite similar levels of angiographic success across the three groups. Obese individuals tended to be younger and have increased levels of hypercholesterolaemia, diabetes, hypertension and higher smoking rates than normal weight individuals (194).

As Gruberg and colleagues point out, their findings were not entirely inconsistent with previous work. The British Regional Heart Study showed a U-shaped relationship between BMI and total mortality in British males aged 40-59 years. The increased mortality in lean males was associated with increased prevalence of debilitating diseases, such as cancer, and with cigarette smoking (195). Cancer has been linked to the development of HF in multiple studies; both reduction in myocardial mass alongside generalised cancer-associated cachexia and cardio-toxic chemotherapeutic agents appear to promote this pathophysiology (196–199).

Smoking is also associated with lower BMI and appetite suppression and studies have shown increased blood glucose associated with smoking cessation (200–202). However, research into blood glucose control in smokers has yielded inconsistent results. Studies have demonstrated both greater blood glucose levels and higher HbA1C levels in smokers relative to non-smokers (203,204). Another study showed transient increase in blood glucose following cigarette smoking was more pronounced in individuals with diabetes than non-diabetics (205). Hence, the inter-relationship between smoking and BMI, appetite and blood glucose levels is still unclear. Greater smoking

prevalence in individuals with HbA1C less than 6% is unlikely to fully explain greater HF incidence observed in this group.

Very few studies have looked at whether hypoglycaemia promotes LVH development.

Hyperinsulinaemic hypoglycaemia has been shown to promote LVH in paediatric patients (206,207).

Research using animal models has shown that drugs blocking the action of insulin-like growth factor-1 are associated with LVH regression (208). Hence, hyperinsulinaemia appears to play a role in LVH development in T2DM. However, association between weighted mean HbA1C<6% and LVH persists even when baseline insulin use is accounted for within the model (table 4.7c). This suggests insulin therapy is not the only causative factor promoting LVH development in this group. However, the mechanism by which relative hypoglycaemia *per se* promotes LVH remains unclear.

5.3.2 Chronic hyperglycaemia promotes HF and LVH development

The progressive non-linear relationship between weighted mean HbA1c $\geq 8\%$ and HF development seen in this study is consistent with previous findings (60,61). Previous observational studies showed HbA1C greater than 7% was associated with increased risk of HF development, where HF was identified through hospital admission data, regardless of CAD status (60,61). These studies used a single measure of HbA1C, which has been shown to underestimate the importance of glycaemic control and calculation of the mean of serial HbA1c measures has been found to be a better predictor of the complications of diabetes (159).

This relationship does not hinge entirely on an association between CAD and glycaemic control.

Weighted mean HbA1C <6% and $\geq 8\%$ were associated with increased risk of HF development regardless of whether CAD was accounted for in the regression model. Weighted mean HbA1C $\geq 7-8\%$ was associated with increased risk of HF development when CAD was excluded from the model, but had no significant effect when CAD was accounted for. This shows that although the association between CAD and hyperglycaemia may contribute to the association between glycaemic control and

HF development, glycaemic control still predicts HF development independently of CAD. This is consistent with findings in the studies described above (209).

A progressive, non-linear, positive relationship was also seen between HbA1C and presence of LVH. Weighted mean HbA1C $\geq 8\%$ was associated with increased LVH prevalence when drugs were not accounted for. This relationship persisted regardless of CAD status. As described above, very little research has examined the relationship between LVH development and HbA1C. However, our results are consistent with previous findings by Sato *et al* (58) who described a progressive increase in prevalence of LVH for every 1% increase in HbA1C outside the normal range (quoted as 4.1–6.4% in their study).

Multiple factors are likely to be involved in the association between chronic hyperglycaemia and both the increased incidence of HF and the increased prevalence of LVH. Sustained hyperglycaemia has been shown to up-regulate the renin-angiotensin-aldosterone system, increase oxidative stress (13,21,23,38,210), promote accumulation of advanced glycation end products and cause interstitial fibrosis and collagen deposition (37,39,40). Additionally, T2DM and hyperglycaemia has been shown to cause myocardial mitochondrial dysfunction, abnormal myocardial substrate metabolism, myocardial lipotoxicity and calcium handling (41,46). These pathophysiological processes are likely to contribute to the associations described above.

5.4 Other factors associated with LVH and HF development

Proportional hazard regression analyses demonstrated increasing age, BMI, creatinine, SBP, presence of CAD and weighted mean HbA1C less than 6% or greater than or equal to 8% were independent predictors of LVH and HF development. Increased DBP was also associated with the development of HF whilst it appeared to be protective against LVH.

The association between HF development in T2DM and age, BMI, creatinine, SBP and DBP and presence of CAD found is consistent with recent work (62). The association between LVH development in T2DM and BMI and renal function is also consistent with previous work (58).

These results are unsurprising given the over-lapping pathophysiology and aetiologies of HF, LVH and T2DM. As described above, multiple studies have demonstrated a link between CAD and HF in T2DM (17,32,33). Hypertension is also a long-recognised risk factor for the development of HF according to observational data from the Framingham study (211,212) and has been shown to promote left ventricular remodelling and LVH in diabetes (25). These findings were supported by later work using UKPDS trial data, where individuals with T2DM were allocated to either tight blood pressure control or standard therapy. Individuals allocated to tight blood pressure control had a significantly reduced risk of HF development (213).

In addition to CAD and hypertension, obesity and renal impairment are also well-established risk factors for LVH and HF. Obese individuals have increased total blood volume creating a high output state that leads to ventricular dilatation and eccentric hypertrophy associated with diastolic dysfunction. Systolic dysfunction may subsequently develop if increased wall stress fails 'to keep pace with' the rate of dilatation (214). Observational work has shown an association between body habitus and left ventricular mass (215) and the increased risk of HF development in obese individuals has also been confirmed in observational studies (19). The cardiovascular consequences of renal impairment, represented in this study by raised creatinine, are an important clinical consideration as cardiovascular disease is the most common cause of mortality in patients with renal failure. Renal function was shown to be an independent predictor of left ventricular mass in patients with T2DM (216). Renal impairment has also been associated with increased risk of HF development and poor outcome in individuals with established HF (217).

The results from the studies described above clearly indicate hypertension, obesity, renal impairment and CAD are important risk factors for the development of LVH and HF in diabetes. Although they are independent risk factors in this study, there is significant interplay between them.

Our results also demonstrated a link between HbA1c variability and the development of HF. Previous reports linked HbA1c variability to the risk of microvascular complications of both type 1 (83) and type 2 diabetes (218). Retrospective analyses of the DCCT (191,218) showed variation in HbA1c was an independent risk factor for the development of both diabetic retinopathy and nephropathy in individuals with type 1 diabetes. In the FinnDiane study, HbA1c variability also predicted incident cardiovascular events (85) although the link with cardiovascular events was not a consistent finding.

We examined the association of intra-individual HbA1c variability and incident HF and showed that lower variability in HbA1c had been relatively protective against incident HF. The physiological mechanisms through which HbA1c variability contributes to HF development are not known. However, increased glucose variability has been reported to have promote cell apoptosis and oxidative stress (42–44). Both these processes can contribute to the pathogenesis of HF (45). The suggestion that HbA1c variability enhances cell apoptosis and oxidative stress in patients with diabetes remains speculative and cannot be concluded from this study.

5.5 Glycaemic control and CAD

It has long been recognised that T2DM is an independent risk factor for the development of CAD (219). However, research looking at whether glycaemic control influences the development of CAD in T2DM has yielded conflicting results. Several studies looking at development of CAD in type 1 diabetes mellitus (T1DM) have shown no relationship between glycaemic control and CAD development. The WESDR study reported no association between glycaemic control, reflected by HbA1C, and development of angina pectoris or myocardial infarction (220). The EDC Study found no association between glycaemic control and the development of CAD in a prospective study of 657 patients with T1DM (221).

Studies looking at glycaemic control in T2DM and CAD development have yielded guarded conclusions. A prospective study of 11644 individuals with T2DM followed up for 4 years showed

hyperglycaemia was an independent risk factor for CAD development in men but not women (222).

A meta-analysis of 5 independent studies looking at whether glycaemic control predicted HF development in T2DM found the overall relative risk was 1.13 (CI 1.06-1.20) for each 1% increase in HbA1C (223).

In the context of the above results, it may be hypothesized that glycaemic control predicted HF and LVH development in our cohort because glycaemic control was associated with CAD, which then led to HF development. The above analyses do not support this hypothesis; the data in tables 3 and 5a showed glycaemic control remained an independent risk factor for HF development when CAD was accounted for in the proportional hazard regression model. Notably, risk associated with differing levels of glycaemic control when CAD was excluded was not significantly different from when CAD was included (Table 5b).

Similarly, glycaemic control was independently associated with LVH development when CAD status was taken into account (see tables 4 and 7a). Furthermore, risk associated with differing levels of glycaemic control when CAD was excluded was not significantly different from when CAD was included (Table 7b). These results strongly suggest neither the relationship between glycaemic control and LVH nor the relationship between glycaemic control and HF development was dependent on CAD status.

5.6 LVH and mortality and morbidity

LVH was associated with increased mortality and hospital admission due to cardiovascular morbidity as illustrated in figure 4.1.4.2. This is consistent with the existing body of evidence showing LVH strongly predicts overall cardiovascular mortality, myocardial infarction, development of heart failure and cerebrovascular events (53,224–226). As described above, LVH is common in T2DM and T2DM is an independent risk factor for LVH (227,228). A large proportion of patients with T2DM and no overt cardiovascular disease have LVH representing a silent population at increased risk of debilitating cardiovascular disease or death. In view of this, screening individuals with T2DM for LVH

may be beneficial. However, primary prevention strategies in individuals with T2DM at the highest risk of LVH may yield the greatest benefit.

Identifying those individuals with T2DM and LVH would only be useful if methods existed to reverse this adverse cardiac remodelling. Recent work has identified multiple potential methods for promoting LVH regression including mineralocorticoid receptor blockade, e.g. using spironolactone (229). Indapamide, felodipine, ACE inhibitors and losartan have also been shown to promote LVH reversal (230–232). Perhaps more surprisingly, high-dose allopurinol has been shown to promote LVH regression according to cardiac MRI performed at both baseline and at 9 months (233). These results were replicated specifically looking at patients with T2DM (234). In view of this, it would arguably be worthwhile to identify individuals with T2DM who are at particularly increased risk of LVH so either a primary prevention or early treatment strategy can be employed.

5.7 Genetic component to LVH in T2DM

A Medline search was performed to identify studies looking at the genetic component to LVH in T2DM but did not identify any studies. It seems the above study represents the first genetics study investigating LVH in T2DM. Our research into the genetics of LVH in T2DM yielded 3 major findings. Firstly, 63% of patients with T2DM undergoing echocardiographic examination had LVH, comparable to previous studies quoting 65% (228). Secondly, the presence of LVH is independently associated with greater mortality in our cohort compared to controls, which is consistent with findings in previous studies (235). Thirdly, in this genetics study of a large population of patients with T2DM in Scotland, SNPs previously identified to be predictors of LVH in the general population were replicated.

Diabetes remains an independent predictor of LVH. However, not all patients with T2DM develop LVH and those developing LVH do so to varying degrees (236), implying LVH in

T2DM has a genetic component. The genetic basis of LVH has been studied in the general population and variants in genes coding for ACE and the beta 1 adrenergic receptor were associated with LVH (237,238). Variants at these loci were not described in the previous GWAS publications looking at LVH and showed no sign of association in our study. This has been a common feature of the transition from candidate gene studies to GWAS, although notable exceptions include the PPARG Pro12Ala variant for T2DM (239).

5.7.1 Considerations in investigating the genetic aspect of LVH in T2DM

Our results suggest susceptibility to LVH in T2DM has a detectable genetic component, though investigating any aspect of LVH is intrinsically difficult as there is still discord regarding the optimum way to measure left ventricular size. Electrocardiography is the least expensive method of detecting LVH but the sensitivity is poor in T2DM; echocardiography is more sensitive (228). Multiple methods of assessing LVH by echocardiography exist including direct 2D measurements, calculation of relative wall thickness and increase in left ventricular mass. Left ventricular mass calculation by echocardiography is the gold standard measure (155,156,225) but complex calculations are involved making direct 2D measurements more popular clinically.

Lack of consensus in defining LVH has also made it difficult to compare genetic findings across studies. It may be argued that the way in which LVH influences outcome is the most important consideration. The results shown above demonstrate the definition employed in this study strongly predicts mortality, showing our method of identifying LVH cases was prognostically useful.

Difficulties in replicating genetic variations predicting LVH development are increased by wide variation in the populations from which cases of LVH were extracted prior to

genotyping. Initial studies looked at LVH familial correlations (36) whilst later work sought variations in candidate genes in hypertensive patients (101,102) in which LVH was associated with variation in the GNB3 gene encoding the G-protein β_3 subunit.

Genetic variations frequently influence more than one disease phenotype. A recent study by Povel *et al* showed SNPs associated with waist circumference (rs17782312 within MC4R) and insulin resistance (rs2943634 within IRS1) were also associated with the metabolic syndrome (240). Genetic variants associated with features such as serum lipid levels, insulin-resistance and waist circumference in T2DM may also be associated with LVH. Additionally, SNPs may interact with one clinical parameter to influence another. Yin and colleagues found variation in 8 SNPs interacted with obesity to influence serum lipid levels but did not influence serum lipid levels in normal weight individuals (241). SNPs may interact with other clinical features, such as obesity and lipid levels, to promote LVH in T2DM. This may be an area for future research.

5.7.2 The role of GWAS and importance of replication

GWAS have broader scope to identify many genetic variants associated with a disease compared to candidate gene studies. However, only two large, population-based GWAS looking at LVH have been performed (105,106). Vasan *et al* compared cases of echocardiographically defined LVH with controls across 5 discovery cohorts (n=12 612) and 2 replication cohorts (n=4 094). Multiple SNPs were linked to LVH, but none were replicated (105). Shah *et al* genotyped 10 256 individuals from 3 population based cohorts, identifying cases of LVH by ECG criteria and comparing to non-LVH controls. Four SNPs associated with LVH at genome wide significance were also significant in their replication cohort (n=11 777,

(106)) but were not significantly associated with LVH in the population based ECHOgen study.

Two out of the nine previously identified SNPs were replicated at the 1 in 20 level, suggesting true replication. Rs17132261 (105), is found near the SLC25A46 gene that codes for a mitochondrial phosphate transporter (242). Its minor allele frequency (MAF) in the general population is 0.11 (243). This proved to be a rare variant within our population (MAF 0.01) and no homozygotes were identified. This was the only SNP that showed any sign of replication for left ventricular thickness in Stage 2 of the study by Vasan *et al*, and is the only SNP replicated from this study. Abnormal myocardial energetics may be significant in cardiac pathophysiology (55), so variation here may logically be associated with LVH.

The second replicated SNP, rs2292462, was published by Shah *et al* (106) and is found in the NMB gene, which has been linked with satiety and weight regulation (244). Rs2292462 has a MAF of 0.34 in the general population (243), with a MAF of 0.45 in our population. The Kaplan- Meier plot in figure 4.1.5.2 shows the guanine base variant at rs229462 effects outcome in an allelic additive manner. Notably, variation in rs2292462 was not predictive of obesity in this study and was associated with LVH when weight was taken into account. This suggests its link with obesity is not driving its association with LVH and adverse outcome in our population.

Rs4966014, within IGF1R, became genome wide significant (3.35×10^{-8}) in the combined meta-analysis after inclusion of our data, despite this not being significant in our dataset.

Rs4966014 was more robustly associated with LVH than any of the others identified by Shah *et al*. The role of IGF1 signalling in cardiac hypertrophy is well established (245,246) and the SNP lies immediately adjacent to a regulatory region recently identified by the ENCODE as

being marked as transcriptional active in muscle cells, by Histone acetylation and DNase 1 sensitivity (245). This illustrates that it is unclear which, if any, of the other loci were not replicated due to a lack of power, or a true difference in the determinants of echocardiographically defined LVH and ECG-LVH.

5.7.3 The genetics of HF development and glycaemic control are inter-twined

Three SNPs previously associated with glycaemic control were also associated with the development of HF in T2DM in our cohort: rs560887, rs7944584 and rs10885122. These SNPs were associated with fasting glucose levels in the GWAS performed by Dupuis and colleagues (140).

5.7.4 Rs560887

Rs560887 lies within the G6PC2 gene on chromosome 2 and its association with glucose homeostasis has been confirmed on multiple studies (247–249). An *et al* replicated this association in a GWAS performed to identify SNPs associated with HbA1c (247). Replication was also achieved in a sub-study of the GLACIER study looking for association between known ‘glycaemic control’ SNPs with fasting glucose and glycaemia 2 hours post-glucose challenge in 16,330 individuals and glycaemic over 10 years in 4,059 of these participants (248). Kelliny and colleagues genotyped over 2 000 healthy children for 16 SNPs associated with glucose homeostasis in adults and found an association between mean fasting glucose and rs560887 (249).

The link between the genetic basis of glycaemic control and cardiac pathology was further strengthened following a Mendelian randomisation study looking at a cohort of 80 522 individuals from Copenhagen, Denmark. The authors assessed how non-fasting glucose

levels were associated with both the development of ischaemic heart disease and SNPs previously associated with glycaemic control. They then assessed whether the replicated SNPs were associated with the development of ischaemic heart disease. Results showed increased non-fasting glucose levels were associated with an increased risk of developing ischaemic heart disease, variation in rs560887 was associated with variation in non-fasting glucose levels and rs560887 was associated with the development of ischaemic heart disease in this cohort (250).

The G6PC2 gene encodes an enzyme that is part of the glucose-6-phosphatase catalytic subunit family, which catalyse the terminal step in both the gluconeogenic and glycogenolytic pathways, integral to the release of glucose into the bloodstream (251). Whilst it clear variation in this enzyme may influence glucose levels, it is less obvious why variation here should promote HF development. Some evidence for a link between gluconeogenesis and HF has been produced through animal models. One study showed gluconeogenesis was increased in mice with HCM (252). Interestingly, progression from hypertension to HF in the rat model of HF created by Kato and colleagues was associated with cardiac cachexia and reduced gluconeogenesis (253).

These may partially explain why Dupuis and colleagues found the A allelic variant of the rs560887 SNP was associated with higher fasting glucose whereas the T variant was associated with HF development and the A variant was relatively protective in the above analysis. Whilst increased gluconeogenesis would promote greater fasting glucose levels, reduced gluconeogenesis may be part of the progression to HF development, as suggested in the model described above. However, this information should be taken in the context of other research showing HF promotes gluconeogenesis (254). The relationship between

glucose homeostasis and HF development is highly complex so it is unsurprising that the underlying pathogenetics are also intricate.

5.7.5 *Rs10885122*

Rs10885122 lies near the ADRA2A gene on chromosome 10. Its association with glucose homeostasis and T2DM has been supported by multiple subsequent studies. Its association with fasting glucose and long-term deterioration in glucose homeostasis was replicated in the sub-study of the GLACIER study as described above for rs560887 (248). Rs10885122 was also used to produce a genotypic-risk score for T2DM development in Japanese individuals (255).

The ADRA2A gene encodes the 2A subtype of the alpha adrenergic receptor, a vital part of the signalling pathway leading to the sympathetic response. Sympathetic over-stimulation is integral to the pathophysiology, so it is logical that variation here may influence the risk of HF development. As part of the sympathetic response, activation of the alpha (2A) adrenergic receptor mediates suppression of insulin secretion in response to glucose levels (256). Variation in other SNPs within the ADRA2A gene have also been associated with glucose homeostasis. Rs553668 is one such SNP; variation here has been shown to influence *in vitro* insulin exocytosis from pancreatic beta islet cells (257). Recent meta-analysis showed variation in rs553668 was associated with risk of T2DM. However, there was no association between T2DM risk and rs10885122 in this analysis (258).

Variations in the ADRA2A gene have also been associated with other cardiovascular risk factors such as propensity to store body fat and tobacco dependence (259,260).

The G variant of the rs10885122 SNP was associated with higher fasting glucose levels in the discovery GWAS. However, in our study, the T variant was associated with HF development and the G variant was relatively protective. This seemingly opposing directionality may be explained by the increased risk of HF development in individuals with relative hypoglycaemia in our analysis described above. It is likely that hyperglycaemia, hypoglycaemia and response to anti-hyperglycaemic drugs have a partial genetic basis. Hence, it is not necessarily surprising that variations in rs560887 and rs10885122 associated with lower fasting glucose levels in previous work were associated with HF in this study.

5.7.6 Rs7944584

Rs7944584 lies near the MADD gene on chromosome 11. Several studies have shown this locus is important in glycaemic control. One study looked at 1782 non-diabetic volunteers at increased risk for T2DM who underwent oral glucose tolerance testing with measurement of insulin, proinsulin and C-peptide levels. Participants were genotyped for at 12 loci for SNPs previously associated with glycaemic control, including rs7944584. The effect allele was associated with raised proinsulin to insulin ratio, indicating impaired conversion of proinsulin to insulin. Variation in rs7944584 was also associated with altered insulin sensitivity (261).

A replication study performed in individuals of Chinese origin provided further confirmation of the association between rs5944584 and glucose homeostasis. Over 3 000 individuals with T2DM and an equal number of controls were genotyped for 12 SNPs previously shown to influence glycaemic traits in people of European descent. Variation at rs5944584 was associated with susceptibility to T2DM and with fasting glucose (262). Li and colleagues produced a knock out mouse to further explore the role of the MADD gene in glycaemic

control. Deletion of IG20/MADD in β -cells resulted in hyperglycaemia and glucose intolerance due to a severe defect in glucose-induced insulin production, supporting the evidence that this locus is likely to be important in glycaemic control and T2DM (263).

The MADD gene encodes the MAP-kinase activating death domain, which is protective against apoptosis in response to TNF α signalling and is highly expressed in neoplastic cells relative to non-neoplastic cells (264). Hence, the majority of work looking at the impact of genetic variation here has focused on its impact on response to chemotherapy and radiotherapy (265,266). Although it is not immediately obvious why variation in this gene should effect susceptibility to HF or influence glycaemic control, it is well recognised that increased TNF α levels are associated with progression of HF (267–269).

TNF α mediates many of the pathological process in HF including vasoconstriction through signalling in vascular smooth muscle cells, mitochondrial dysfunction, increased oxidative stress and myocyte apoptosis (270,271). Early research into the cellular mechanisms underlying HF showed increased apoptosis correlated with reduced cardiac output and increased left ventricular end-diastolic pressure in a canine model of HF (272) and even low levels of myocyte apoptosis in a mouse model was associated with the development of dilated cardiomyopathy (273). The importance of TNF α mediated myocyte apoptosis was later confirmed in human studies (274).

Thus, there is plentiful evidence that TNF α partially promotes HF development and progression through increasing cardiac myocyte apoptosis. Taking this into account, it is understandable that genetic variants that inhibit TNF α mediated apoptosis may influence the development of HF. This may partially explain the association between variations in rs7944584, near the MADD gene, and the development of HF.

Unlike the other 2 replicated SNPs, the directionality of the association between rs7944584 and fasting glucose in the discovery GWAS was the same as the directionality its association with HF development. Specifically, the T variant of the rs7944584 SNP was associated with lower fasting glucose levels in the discovery GWAS and protective against HF development in our cohort. This may be explained through the association between hyperglycaemia, regarded as weighted mean HbA1c >8%, and HF development in our study.

5.8 Study limitations

Inherent limitations accompany a retrospective, non-randomised observational study. Firstly, values used for covariates were taken at regular clinical visits rather than at the time of echocardiography. Baseline values for BMI, SBP, DBP, creatinine, age and diabetes duration were used for the survival analysis looking at how weighted mean HbA1C influences HF development. However, in the logistic regression analyses looking at the genetics of LVH and the genetics of hypoglycaemia, values closest to the date the echocardiogram was performed were used for cases of LVH and values closest to genotyping date were used for the control subjects. Arguably, if there had been changes in these covariates between the 2 time points in either of the above analyses, it would have biased our results towards the null.

Secondly, we were unable to model use of anti-hyperglycaemic medication time-dependently and so cannot determine whether the association between anti-hyperglycaemic medications and HF or LVH development depended on medication-dose or the duration of drug exposure. Many patients are on oral anti-hyperglycaemic agents for many years before commencing insulin therapy, the cumulative exposure of these agents

was not taken into account. Randomized controlled trials would be the least biased way to look at the influence of anti-hyperglycaemic medications in the development of HF and LVH. However, it is questionable whether such trials would be ethical given the results of previous RCTs looking at outcome in intensive versus standard glycaemic control (63–65).

Thirdly, the retrospective nature of our study meant we did not have echocardiographic data for our non-LVH controls or non-HF type 2 diabetics. Our identification of non-LVH controls was based on the hypothesis patients undergoing echocardiography for any reasons were more likely to have genetically-promoted structural cardiac pathology.

Patients on loop diuretics were excluded from both the non-LVH control population and the non-HF population. These patients were deemed more likely to have fluid retention related to either undiagnosed LVH with diastolic dysfunction or undiagnosed LV systolic dysfunction. The clear difference in mortality between cases of LVH and non-LVH controls supports the method used to identify non-LVH controls, as does the fact that we replicated SNPs previously associated with LVH.

Fourthly, the sample size in this study was small relative to previous studies. In the LVH analysis, although only 2 previously published SNPs were replicated, 4 published SNPs retained genome wide significance following meta-analysis with our data. This may indicate insufficient power to replicate the other 2 SNPs using the data available.

Fifthly, whilst multiple covariates were included in the above analyses, some potentially relevant covariates were omitted due to lack of data, reflecting the limitations imposed by the retrospective nature of the study. Previous studies have shown smoking status to be

relevant in the development of HF and LVH and that tachycardia also promotes HF (275–277).

Sixthly, all the genetic typing data available for these analyses was for individuals taking statins. This is a potential source of bias as there is a greater probability that this group of individuals had pre-existing vascular disease or were high risk. This should be considered when interpreting the results.

Chapter 6: Conclusions

The results discussed above support the hypotheses described earlier:

- 1) Glycaemic control is an independent risk factor for the development of LVH and HF rather than promoting LVH and HF indirectly through other pathological processes such as CAD
- 2) Overly strict glycaemic control, specifically weighted mean HbA1C < 6%, promotes the development of HF and LVH in T2DM. According to the above results, the optimum range for glycaemic control appears to be weighted mean HbA1C 6–8% with respect to development of LVH and HF in T2DM
- 3) Greater intra-individual variation in glycaemic control is an independent risk factor for the development of HF in T2DM
- 4) There is a partial genetic basis for the development of LVH in T2DM, which overlaps with the genetic basis of LVH in the general population
- 5) Genetic factors predicting glycaemic control also appear to influence the development of LVH and HF in T2DM

Publications

Parry HM, Donnelly LA, van Zuydam N, Doney ASF, Elder DHJ, Morris AD, Struthers AD, Palmer CNA, Lang CC. Genetic variants predicting left ventricular hypertrophy in a diabetic population: a Go-DARTS study including meta-analysis. *Cardiovascular Diabetology*, 2013, 12:109

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